Biochimica et Biophysica Acta, 552 (1979) 413–417

BBA 78336

DIMINISHED CEREBROSIDE-SULFOTRANSFERASE ACTIVITY IN THE JIMPY MOUSE MUTANT DUE TO ALTERED LIPID COMPOSITION IN MICROSOMAL MEMBRANES

H. JUTZI, H.P. SIEGRIST *, T. BURKART, U. WIESMANN and N.N. HERSCHKOWITZ

Department of Pediatrics, University of Berne, 3010 Berne (Switzerland)

(Received September 18th, 1978)

Key words: Sulfatide metabolism; Metachromatic leukodystrophy; Cerebroside-sulfotransferase; (Jimpy mouse)

Summary

The mouse mutant Jimpy shows a deficient myelination. In the microsomes of the Jimpy brain, the cerebroside-sulfotransferase (EC 2.8.2.11) activity is low.

The cerebroside-sulfotransferase activity of Jimpy microsomes could be normalised by delipidating the microsomes with cold acetone and adding to them acetone-extracted lipids from normal microsomes. The lipids extracted from Jimpy membranes did not influence the cerebroside-sulfotransferase activity of neither normal nor Jimpy microsomes. The same results were obtained if artificial cholesterol-phospholipid mixtures in ratios corresponding to the ones found in normal and Jimpy membranes were used for recombination experiments. Therefore the diminished enzyme activities in Jimpy microsomes may be related to the lowered cholesterol-phospholipid ratio found in the microsomal membranes of the Jimpy mutant.

Introduction

In the central nervous system of the Jimpy mouse mutant myelination is greatly impaired, due to a still unknown primary defect [1]. However, one of the factors involved seems to be a decreased synthesis of myelin glycolipids [2] such as sulfogalactosylceramide (sulfatide). Compared to microsomes from normal mouse brain, the activity of 3'-phosphoadenosyl-5'-phosphosulfate-galactosylceramide-sulfotransferase (cerebroside-sulfotransferase) in Jimpy microsomes is diminished to a value of 30% [3,4]. This microsomal enzyme

* To whom correspondence should be sent.

catalyses the transfer of a sulfate group to galactosylceramide (cerebroside) [5]. It shows a lipid requirement [6], which leads to a modulation effect [7]. In the mutant, the physicochemical properties of the enzyme seem to be normal [8]. It could, therefore be that the lowered enzyme activity in the mutant is due to a changed lipid pattern of the microsomal membranes. Since it is known that cholesterol synthesis in the Jimpy brain is reduced [9], we checked in this study whether this abnormal cholesterol content and the concomitantly changed cholesterol/phospholipid patterns could be responsible for the reduced activity of cerebroside-sulfotransferase in Jimpy mouse brain microsomes.

Materials and Methods

Jimpy mutants (C57BL/6J-W A-J) and their normal littermates were purchased from Jackson-Laboratories, Bar Harbor, ME, U.S.A. and have since been inbred in our laboratory. Bovine cerebroside, phosphatidylcholine and cholesterol were from Sigma chemicals, sodium deoxycholate from Merck and 3-phosphoadenosine-5′-phospho [^{35}S]sulfate [^{35}S]PAPS and Permablend II from NEN Radiochemicals, F.R.G.

Tissue preparation. Mouse brains were obtained from 18-day-old male animals after decapitation. Total brains were weighed and immiedately homogenized in 9 vols. of cold 0.32 M sucrose, containing 20 mM EDTA and 2.5 mM Na_2HPO_4 [10]. The homogenate was centrifuged at 17 000 × *g* for 20 min in an ultracentrifuge MSE superspeed 65, Titan fixed angle rotor TW 65, (MSE Ltd. London, UK).

The supernatant was decanted by suction and then centrifuged at 105 000 × *g* for 90 min. This pellet contains the microsomal cerebroside-sulfotransferase activity [7].

Acetone extraction. 10—12 mg of microsomal pellet was resuspended in 1 ml 0.9% NaCl by homogenization and transferred to a glass tube containing 9 ml acetone. The suspension was stirred again for 1 min and centrifuged at 3000 × *g* for 3 min. The pellet was washed twice with precooled acetone and dried under a stream of nitrogen. The whole preparation was carried out at —20° C [7].

Preparation of liposomes. The acetone supernatant containing the original lipids was dried by a stream of nitrogen in a glass-teflon homogenizer. After addition of an appropriate amount of 0.9% NaCl the suspension was homogenized and sonicated for 3 min in a sonication bath. In order to prepare model lipid mixtures, cholesterol and phosphatidylcholine (chain length 18 : 0, Sigma) in adequate amounts were dissolved in chloroform/methanol (2 : 1, v/v) and dried in a glass-teflon homogenizer. After addition of 0.9% NaCl the suspension was homogenized and sonicated for 3 min in a sonication bath.

Incubation conditions. Incubation was carried out as described previously [7]: 100 μg of delipidated microsomal protein were incubated with 100 μg of bovine cerebrosides (Sigma) and 300 000 dpm [^{35}S]PAPS (NEN). The total volume was 600 μl, incubation was carried out for 20 min at 30° C at a pH value of 7.0. The lipid/protein ratio for recombination experiments was kept as found in intact microsomes (1.2 for normal and 0.9 for Jimpy microsomes). As a detergent 0.01% sodium deoxycholate was added.

Analytical procedure. Cholesterol was determined in lipid extracts according

to Folch-Pi et al. [10] by the methods described by Herschkowitz et al. [11]. Phospholipid determination was done by the method of Donaldson et al. [12]. Protein determination was carried out according to the method of Lowry et al. [13]. Bovine serum was used as a standard.

Results and Discussion

In a first series of experiments, microsomes from 18-day-old mouse brains (normal and Jimpy) were delipidated with cold acetone and incubated in all combinations with their acetone-extracted lipids. The results presented in Table I show that the delipidation process reduces the enzyme activity of normal microsomes down to 30% of intact microsomes of 18-day-old animals, but has no effect on the microsomal enzyme in Jimpy, which even before treatment with acetone has an enzyme activity of only 30% if compared to normal microsomes.

The readdition of acetone-extracted lipids restores the activity in normal microsomes and stimulates the Jimpy preparations to a value of 80% of normal. On the other hand, lipid preparations derived from Jimpy microsomes do not show any effect either on Jimpy or on normal microsomes. The results confirm the observation made in an earlier study that normal as well as Jimpy cerebroside-sulfotransferase shows the same lipid requirement [6]. However, those studies were based on experiments carried out with a solubilized enzyme preparation, and it seems likely that those results do not reflect the situation in the intact membrane. In order to study the enzyme in its native situation, in the present study we used an assay in which the 0.5% Triton X-100 concentration is replaced by 0.01% sodium deoxycholate, a detergent concentration which is below the critical micellar concentration. This detergent concentration does not influence neither membrane properties [14] nor recombination effects [7]. Due to the lowered cholesterol content, in 18-day-old Jimpy brain

TABLE I

SPECIFIC CEREBROSIDE-SULFOTRANSFERASE ACTIVITIES OF INTACT MICROSOMES, ACETONE-TREATED MICROSOMES AND ACETONE-TREATED MICROSOMES FROM 18-DAY-OLD NORMALS AND JIMPY RECOMBINED WITH THEIR ACETONE-EXTRACTED LIPIDS

Delipidation and recombination was carried out as described in Materials and Methods. The values are means of three independent experiments ± S.E. Differences between normal and Jimpy lipid reactivation are statistically significant ($P < 0.01$).

Microsomes	Source of acetone extracted lipids	Enzyme activity (dpm [^{35}S]sulfatide per mg protein)
Normal intact	—	9326 ± 1100
Jimpy intact	—	2970 ± 540
Normal acetone treated	—	2313 ± 420
Jimpy acetone treated	—	2460 ± 560
Normal acetone treated	Jimpy	3911 ± 416
Jimpy acetone treated	Jimpy	3328 ± 882
Normal acetone treated	normal	8155 ± 1081
Jimpy acetone treated	normal	7572 ± 915

TABLE II

SPECIFIC CEREBROSIDE-SULFOTRANSFERASE ACTIVITIES OF ACETONE-DELIPIDATED MICROSOMES FROM 18-DAY-OLD JIMPY RECOMBINED WITH MODEL LIPID MIXTURES CONTAINING CHOLESTEROL AND PHOSPHATIDYLCHOLINE IN VARYING RATIOS

The values are means of four independent experiments with 4—5 male animals. Delipidation of microsomes with acetone, preparation of the artificial lipid mixture and reactivation of the enzyme was carried out as described in Materials and Methods. Values are expressed as means ± S.E.

Cholesterol/phospholipid ratio of the model lipid mixtures used for recombination	Enzyme-activity (dpm [^{35}S]sulfatide per mg protein)
0.60	7 210 ± 820
0.54	10 250 ± 490
0.47	7 510 ± 790
0.40	3 480 ± 780

microsomes the cholesterol/phospholipid ratio is decreased to a value of 0.40. This is in contrast to 18-day-old normal microsomes (used as controls), where a ratio of 0.54 is found. A cholesterol/phospholipid ratio of 0.40 should lead to an enzyme activity of about 40% of that of normal 18-day-old microsomes [7] as shown for the age dependent enzyme modulation before. Since this value corresponds well to the one found in this study for 18-day-old Jimpy microsomes, we studied in a next step the influence of different cholesterol/phospholipid ratios on an acetone-treated microsomal enzyme preparation of 18-day-old Jimpy brains. As shown in Table II, a maximal stimulation rate is found for a cholesterol/phospholipid ratio of 0.54, corresponding to 18-day-old normal microsomes. A ratio of 0.60 as found in 10-day-old normal animals, leads to a decrease of activity as does a value of 0.40, which corresponds to 25-day-old normal animals or, as shown, 18-day-old Jimpy brains. In summary it can be said that the enzyme activity in Jimpy microsomes is modulated in the same manner as the activity of normal brains [7] and can be corrected by adding cholesterol/phospholipid mixtures in the adequate ratio to acetone-treated microsomes. In order to avoid an artifact produced by the subfractionation of normal and Jimpy mouse brains which differ in their myelin content, the recombination experiments were reproduced using whole brain homogenate instead of microsomes. Thereby we found that recombination of acetone-treated Jimpy brain homogenate with an adequate amount of acetone-extracted lipids from normal brain homogenate resulted in an activity of 200 000 ± 15 090 dpm (S.E.) [^{35}S]sulfatide per mouse brain. This means an increase to a value which corresponds to 83 ± 12% (S.E.) of normal homogenate. If this delipidated homogenate was recombined, the model lipid mixture (cholesterol/phospholipid ratio of 0.54) an activity of 237 740 ± 16 980 dpm [^{35}S]sulfatide per mouse brain could be observed. This means 98 ± 14% (S.E.) of the homogenate from normal mouse brain. Delipidated homogenates from Jimpy and normals showed an activity of 29 ± 9 and 23 ± 6%, respectively, of homogenate from normal mouse brain. We postulate that also in Jimpy brains the activity of cerebroside-sulfotransferase is controlled by the lipid composition of the microsomal membrane and that the decrease in activity is due to the lowered cholesterol content which changes the cholesterol/phospholipid ratio

in these membranes. Our studies have shown, that model lipid mixtures of varying cholesterol/phospholipid composition are able to modulate the enzyme activity in a way which mimics a developmental enzyme activity pattern [7] and, as demonstrated in this paper, normalises the activity of a mutant if the cholesterol/phospholipid ratio is normalized. These findings suggest, that the diminished enzyme activity in Jimpy might be a secondary phenomenon due to an altered lipid composition of the microsomes of this mutant, probably caused by the reduced cholesterol synthesis in the central nervous system of the Jimpy mutant [9].

Acknowledgement

This work was supported by grants number 3.660.075 and 3.768.076 of the Swiss National Science Foundation.

References

1 Sidman, R.L., Appel, S.H. and Fuller, J.F. (1968) Science 150, 513—516
2 Matthieu, J.M., Widmer, S. and Herschkowitz, N.N. (1973) Brain Res. 55, 403—412
3 Herschkowitz, N.N., Vassella, F. and Bischoff, A. (1971) J. Neurochem. 18, 1361—1362
4 Sarlieve, L.L., Neskovic, N.M., Rebel, G. and Mandel, P. (1972) Neurobiology 2, 70—82
5 McKhann, G.M. and Ho, W. (1967) J. Neurochem. 14, 717—721
6 Siegrist, H.P., Burkart, T., Steck, A.J., Wiesmann, U. and Herschkowitz, N.N. (1976) J. Neurochem. 27, 599—604
7 Siegrist, H.P., Jutzi, H., Steck, A.J., Burkart, T., Wiesmann, U. and Herschkowitz, N. (1977) Biochim. Biophys. Acta 489, 58—63
8 Matthieu, J.M., Schneider, U. and Herschkowitz, N.N. (1972) Brain Res. 42, 433—439
9 Kandutsch, A.A. and Saucier, S.E. (1969) Arch. Biochem. Biophys. 135, 201—208
10 Folch-Pi, J., Lees, M. and Sloane-Stanley, G.M. (1957) J. Biol. Chem. 226, 497—509
11 Herschkowitz, N.N., McKhann, G.H. and Shooter, E.M. (1968) J. Neurochem. 15, 161—168
12 Donaldson, R.P., Tolbet, N.E. and Schnorrenberger, C. (1972) J. Biol. Chem. 226, 497—509
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79