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# LIPID COMPOSITION OF SCIATIC NERVE FROM DYSMYELINATING TREMBLER MOUSE

S. LARROUQUÈRE-RÉGNIER, F. BOIRON, D. DARRIET, C. CASSAGNE and J.M. BOURRE

*Laboratoire de Biochimie BBC, Université de Bordeaux II, CNRS - ERA 403, 351 cours de la Libération, 33405 Talence Cedex and (J.M.B.) Laboratoire de Neurochimie, INSERM U-134, CNRS - ERA 421, Hôpital de la Salpêtriere, 75634 Paris Cedex 13 (France)*

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SUMMARY

The amounts and the distribution of the various lipids were studied in the sciatic nerves from normal and trembler mice. When compared to the normal, the total lipidic amount was reduced by 66% in the trembler mouse, and each class of lipid was decreased nearly the same way, except for the cholesterol esters the value of which increased five times.

The level of each individual phospholipid was decreased, phosphatidylcholine being the least affected and phosphatidylserine the most altered.

The trembler mouse [10] is characterised by a dominantly inherited chronic hypertrophic neuropathy [2]. Axons are thinly myelinated and surrounded by very few myelin lamellae which are often uncompact and with frequent abnormal periodicity. Well developed onion bulb formations consisting of thinly myelinated axons surrounded by empty membranal configuration, are often seen [15,16]. This pathological dysmyelination is correlated with electrophysiological abnormalities [18] and alterations in the X-ray diffraction pattern [14].

The presence of normal axons associated with reactive changes in Schwann cells suggest that Schwann cell abnormality is primary and not secondary due to an axonal disorder [3]. Experiments using nerve grafts have unequivocally proved that the trembler mouse neuropathy is due to a primary disorder of Schwann cell [1].

In contrast with peripheral nerve, histological examinations of the brain of the trembler revealed no lesion [2,6]. The pathological changes in trembler have been considered to resemble those of human hypertrophic neuropathies, especially Dejerine-Sottas neuropathy.

The incomplete formation of myelin is biochemically correlated with the reduction of alkanes [8] these molecules being considered as good markers for myelin [5,9] and synthesised by the peripheral nerve [7]. As no extensive biochemical analysis in trembler mouse has been performed, this study was undertaken to determine the lipid composition of sciatic nerve in trembler as compared to normal animal.

Fifty trembler were obtained on B6-CBA strain. Sciatic nerves were carefully removed from neurological mutants and control mice of the same age (adult) and similar body weight. The weight of fresh and lyophilised nerve was determined. Fresh material (at least 12 sciatic nerves) was homogenised in 5 ml chloroform/methanol (1 : 1) (lyophilised samples are suspended in chloroform/methanol/water, 70 : 3 : 4). The extraction was made (according to Folch et al. [11,12] as modified by Pollet al. [19]) by six 30-sec sonications and *stirring for 1 h* and allowed a complete lipid recovery.

The residue was easily pelleted after centrifugation at 2000 rev./min for 10 min; the lipid extract was diluted to a known final volume. An aliquot was evaporated to dryness to determine the weight of the total lipid extract. By using the whole lipid extract, the galactolipids were assayed from galactose determination, the phospholipids by the phosphorus content and Lipid NeuNac by a specific method [19]. The ratio between cholesterol and cholesterol esters was determined on the total lipidic extract by quantitative measurements after thin-layer chromatography with *n*-hexane/ether/acetic acid (90 : 10 : 1) [13].

For quantification of the different lipids within each class, separation was performed on TLC glass plates (10 × 10 cm) coated with silicagel 60 F 254 Merck. About 500 μg of lipid extract were spotted at 2 cm from the bottom and the left of the plate [5,17]. The solvents used in succession were the following: (i) chloroform/methanol/water (70 : 30 : 4) migrating from the bottom to the top of the plate; (ii) at right angle to the first, chloroform/methanol (2 : 8) on 2/3 of the plate; (iii) in the same direction, chloroform/methanol (2 : 1); (iv) finally, chloroform/methanol (2 : 1) in the same direction as that from the bottom to the top of the plate.

Lipids were detected by iodine vapors. After complete sublimation of the iodine, the areas of individual lipids were scraped off and submitted to quantification. So as to eliminate interferences, each assay has been performed with a constant amount of lipid to which were added increasing amounts of the standard substance to be determined. The slope of the curve obtained is compared to one consisting of the standard scale only. This method is called 'standard additions method'. Most of the methods used for cerebrosides, sulfatides, gangliosides and the various phospholipids are classical [19].

Dry weight of the sciatic nerve (Table I) is 32% of the fresh weight in the normal but only about 20% for the trembler; this difference is possibly explained by the severe hypomyelination [8]. The amount of total lipids in the trembler is largely reduced, being 33% of the normal if based upon fresh weight (56% if reported to the lyophylised tissue). Thus, trembler nerve is lipid poor, all the lipids being reduced in nearly the same way.

TABLE I

LIPID COMPOSITION OF SCIATIC NERVE

Cholesterol, cholesterol esters, phospholipids, glycolipids, NANA were assayed on the whole lipidic extract *Number of experiments. Numbers into brackets give the values of each class of lipid in percent of total lipids.

| | Sciatic nerves of | |
|---|---|---|
| | Normal mice 7* | Trembler mice 4* |
| Dry weight/fresh weight | 32.2 | 20.5 |
| Weight of lipid extract (mg/100 mg FW) | 18.0 | 6.0 |
| Weight of lipid extract (mg/100 mg DW) | 54.0 | 30.0 |
| Dosage of lipids (mg/g FW) | | |
| Total of cholesterol | 32.0 (17.8) | 7.3 (12.1) |
| Cholesterol | 31.7 (17.6) | 5.8 ( 9.6) |
| Cholesterol esters | 0.3 ( 0.2) | 1.5 ( 2.5) |
| Phospholipids | 2.1 (29.2) | 0.6 (25.0) |
| Glycolipids | 36.8 (20.4) | 9.6 (16.0) |
| NANA | 0.42 ( 0.2) | 0.26 ( 0.4) |

TABLE II

PHOSPHOLIPIDS AND GLYCOLIPIDS

Each individual lipid was assayed after separation by multidimensional CCM as described earlier.

| Glycolipids | Normal mice | | Trembler mice | |
|---|---|---|---|---|
| | mg/g FW | % of total lipids | mg/g FW | % of total lipids |
| Cerebrosides | 24.2 | 13.5 | 5.7 | 9.5 |
| Sulfatides | 10.1 | 5.6 | 3.2 | 5.3 |
| Gangliosides | 2.2 | 1.2 | 0.7 | 1.2 |
| **Phospholipids** | **mgP/g FW** | **% of total lipids** | **mgP/g FW** | **% of total lipids** |
| Sphingomyelin | 0.35 | 4.8 | 0.11 | 3.9 |
| Phosphatidyl-serin | 0.34 | 4.7 | 0.06 | 2.5 |
| Phosphatidyl-cholin | 0.62 | 8.6 | 0.24 | 10.3 |
| Phosphatidyl-ethanolamin | 0.67 | 9.3 | 0.16 | 7.0 |
| Phosphatidyl-inositol | 0.09 | 1.3 | 0.02 | 0.9 |
| Phosphatidic acid | 0.03 | 0.5 | 0.01 | 0.5 |

The quantification of cholesterol esters shows an increase of the ester amount from 1% of total cholesterol (normal mouse) up to 25% (trembler mouse). Quantification of sugars shows (Table I) that glycolipids are reduced.

Cerebrosides considered as good markers for myelin membrane are slightly more decreased if compared to sulfatides and gangliosides (Table II). As reduction is not similar in gangliosides when measuring either sugars or NANA, the ratio of neuraminic acid over sugar, is probably affected in the trembler sciatic nerve.

Individual phospholipids analysis shows (Table II) that the pattern is altered in the trembler. When considering the percentage, phosphatidylserine is largely reduced and phosphatidylcholine is increased, the values for the others being similar. When considering the amount of phosphorus of each phospholipid reported to the nerve fresh weight, all phospholipids are largely reduced, PS being most affected.

In conclusion, all the lipids except for cholesterol esters are reduced in the trembler sciatic nerves, some lipids being more affected as compared to others. This mutant would be a good model for studying peripheral human hypomyelination as simple and reproducible values are present to analyse the major lipid classes in human peripheral nerve biopsies [20].

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