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EVIDENCE OF AN ABNORMAL STEAROYL-CoA HYDROLYSIS IN A MEMBRANE PELLET FROM TREMBLER SCIATIC NERVES

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A membrane fraction from sciatic nerves of Trembler mouse hydrolyzed stearoyl-CoA, 2–3 times more efficiently than the controls. The maximal hydrolysis within 60 min could reach 40% of the added substrate as compared to 20% in the control. Stearoyl-CoA hydrolysis increased as a function of time (up to 60 min), protein amount (up to 200 μg) and substrate concentration. Addition of ATP and Mg^{2+} markedly lowered the free fatty acid release in the membrane pellet from Trembler mouse sciatic nerves.

The incomplete formation of PNS myelin of the Trembler mouse is biochemically correlated with a reduction of alkanes [6], phospholipids and glycolipids [9]; also, sulfatide biosynthesis is found increased in both total nerve [14] and Schwann cell culture [6].

A two-fold decrease of the total fatty acid amount is also observed, but it does not affect equally the various classes of fatty acids: whereas the monounsaturated fatty acids amount is nearly normal, the saturated C20 to C24 fatty acids represent less than 10% of the normal value [9].

However, a stearoyl-CoA elongase is present in a membrane fraction from Trembler sciatic nerves. The rate of C20 to C24 fatty acid formation reaches only one-third of the control; direct and indirect evidence suggested that a stearoyl-CoA hydrolysis occurred [5,7].

Because of its importance in understanding some aspects of the very long chain fatty acid (VLCFA) biosynthesis by normal and Trembler sciatic nerves, the fate of stearoyl-CoA, substrate of the elongase, was further investigated. This paper reports a comparative study of the stearoyl-CoA hydrolysis by a particulate fraction from Trembler and normal sciatic nerves.

Stearoyl-CoA, ATP, creatine phosphokinase and phosphocreatine were obtained from Sigma and [1-^{14}C]stearoyl-CoA (49 nCi/nmol) was from NEN or from Amersham International Limited.

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Trembler mice were obtained of B6-CBA strain. Controls were the mice with normal phenotype of the same littermate. The sciatic nerves of Trembler and control mice of the same age (30 days) and similar body weight were carefully removed and homogenized in a glass-glass tissue grinder with 10 vol. 0.05 M Tris-HCl, pH 7.5 at 4°C. The homogenates were spun at 20,000 *g* for 20 min and the resulting supernatants were centrifuged at 150,000 *g* for 90 min. The resulting membrane pellet was resuspended in 0.05 M Tris-HCl, pH 7.5, gently homogenized in a Potter homogenizer and used as enzyme source. The proteins were estimated by the Lowry et al. method [13].

Stearoyl-CoA thioesterase (EC 3.1.2.2) was assayed as described previously [5] by an original experimental procedure which led to a rapid and accurate determination of the free fatty acid fraction and allowed a direct measurement of the remaining acyl-CoAs and/or polar lipids in a single step. It was done in Tris buffer 50 mM according to Barden and Cleland [3] in rat microsomes, at pH 7.5 in good agreement with previous studies on acyl-CoA thioesterase which showed a maximal activity at pH 7.4 [10] and pH 7.7 [2] for rat brain, or pH 7.8–8.2 [4] for rat liver.

Occurrence of a membrane stearoyl-CoA thioesterase activity. Within 60 min, the average hydrolysis of the acyl-CoA was about 3 times higher with the Trembler crude membrane fraction as compared to normal.

The dependency of stearoyl-CoA hydrolysis on the pH was very similar for normal and Trembler 150,000 *g* pellets, except that, in the latter case, the level of hydrolysis reached 40% of the added substrate between pH 7 and pH 8 as compared to about 17% in normal membrane fraction.

As the VLCFA synthesis is maximal around pH 7.5, all the subsequent studies were performed at pH 7.5 in order to check if a possible involvement of that hydrolyzing activity could explain the lower VLCFA biosynthesis in Trembler as compared to Normal.

The stearoyl-CoA hydrolysis in 60 min was dependent upon the amount of membrane proteins (Table I). It increased linearly as a function of the protein amount from normal or Trembler 150,000 *g* pellet. However, the increase was higher in Trembler (Tr) than in normal (N), as the ratio Tr/N (which was 1.6 for 25 μg of

TABLE I

STEAROYL-CoA HYDROLYSIS AS A FUNCTION OF MICROSOMAL PROTEIN AMOUNT

Each reaction mixture (60 min, 37°C) contained 20 μM stearoyl-CoA and variable amounts of membrane proteins in a final volume of 0.1 ml. Results are given as the percentage of hydrolysis of administered stearoyl-CoA.

Membrane proteins (μg)	25	50	75	100	150	200
Normal	11.5	12	13	13.7	15.5	17
Trembler	18.5	22	24.5	27.5	32.5	37.5

TABLE II

DEPENDENCE OF THE STEAROYL-CoA THIOESTERASE ACTIVITY ON THE STEAROYL-CoA CONCENTRATION

The assays (37°C, 60 min) contained 150 μg of membrane proteins (for Normal) or 50 μg of membrane proteins (for Trembler). The results are given as nmol of stearoyl-CoA hydrolyzed per mg protein per hour.

Stearoyl-CoA concentration (μM)	5	10	25	50	75	100
Normal	0.1	0.65	2.8	5.8	7.8	7
Trembler	0.5	3	12.5	34	53	66

proteins) reached 2.2 for 200 μg of proteins in similar experimental conditions.

Stearoyl-CoA hydrolysis and stearoyl-CoA concentration. The stearoyl-CoA hydrolysis in the Trembler sciatic nerves membrane pellet increased as a function of stearoyl-CoA concentration. Neither a plateau nor an inhibition of the thioesterase activity was observed, even at the highest acyl-CoA concentration (100 μM stearoyl-CoA) (Table II).

In contrast, with the control membrane pellet there was a maximum of hydrolysis for 75 μM of stearoyl-CoA.

Time course of stearoyl-CoA hydrolysis. The kinetics of stearoyl-CoA hydrolysis were studied between 0 and 60 min, the latter being required for maximal stearoyl-CoA elongation.

As no saturation of the acyl-CoA thioesterase by stearoyl-CoA was observed in the membrane fraction from Trembler mice sciatic nerves up to 100 μM stearoyl-CoA, the kinetics were studied at 75 μM, which is the concentration required for the maximal hydrolysis in 60 min by the membrane fraction from normal sciatic nerves.

Between 0 and 60 min stearoyl-CoA hydrolysis was 1.5 to nearly 3 times higher in the membrane fraction from Trembler mouse sciatic nerves than in the controls (Table III).

TABLE III

TIME COURSE OF STEAROYL-CoA HYDROLYSIS BY THE MEMBRANE FRACTIONS FROM TREMBLER AND NORMAL SCIATIC NERVES

Each reaction mixture (37°C) contained 75 μM stearoyl-CoA and 50 μg of normal or Trembler membrane fraction. The results are given as the percentage of hydrolysis of administered stearoyl-CoA.

Time (min)	2	5	10	30	60
Normal	3.5	4.3	5	7.2	11.5
Trembler	4.5	6.3	12.3	19.5	30

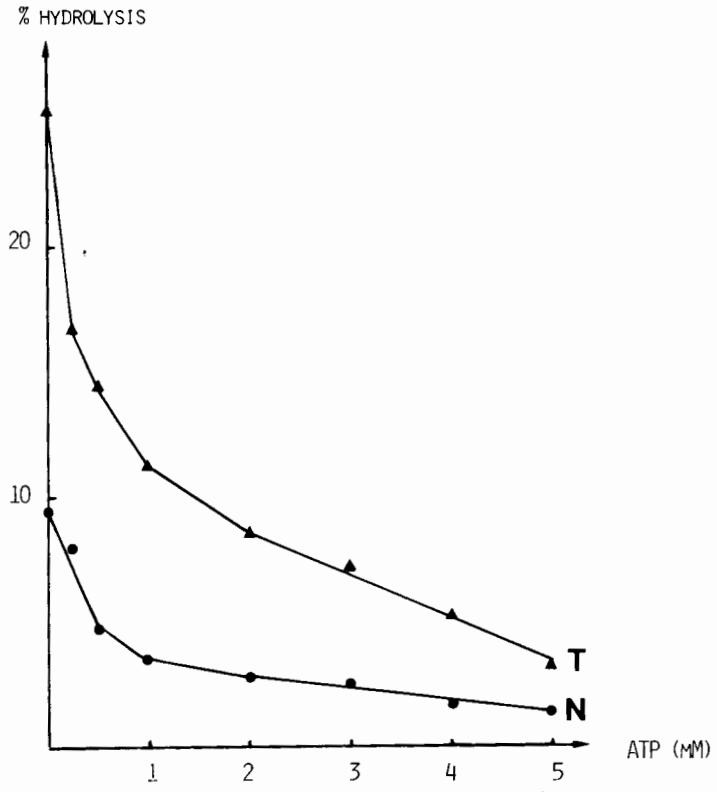

Fig. 1. Effect of ATP–Mg^{2+} concentration on the stearoyl-CoA thioesterase activity. The reaction mixture contained 50 μg of membrane proteins from Trembler (T) or normal (N) mice sciatic nerves, 50 μM stearoyl-CoA. 0 to 5 mM ATP–Mg^{2+} was added to the reaction mixture in the presence of creatine phosphokinase (0.15 mg/assay) and 20 mM creatine phosphate. The results are given as the percentage of hydrolysis of administered stearoyl-CoA as a function of the amount of ATP–Mg^{2+}.

Effect of an ATP–Mg^{2+} addition on the stearoyl-CoA hydrolysis. An ATP–Mg^{2+} addition (20–50 mM) was able to reduce markedly the stearoyl-CoA hydrolysis. The high levels of required ATP were due to the hydrolysis of this substrate in the Trembler membrane fraction; this was demonstrated by chromatography of the reaction mixture (50 μl/assay) on PEI-cellulose plates eluted by 1 mM LiCl after 1 h incubation and for increasing concentrations of ATP–Mg^{2+} (between 2 and 50 mM).

Thus, the effect of increasing amounts of ATP–Mg^{2+} on the stearoyl-CoA hydrolysis was checked in the presence of an ATP regenerating system (creatine phosphokinase 0.15 mg/assay; phosphocreatine 20 mM).

Whatever the ATP–Mg^{2+} concentration up to 5 mM, its addition resulted in a decrease of the stearoyl-CoA hydrolysis by the membrane fractions from Trembler and normal mice sciatic nerves (Fig. 1).

A 5 mM ATP–Mg^{2+} addition resulted in an almost identical level of stearoyl-CoA hydrolysis in both Trembler and normal particulate fractions.

Our results demonstrate that the membrane fraction from Trembler mice sciatic nerves exhibits an abnormally high stearoyl-CoA hydrolyzing activity: whatever the pH, time of incubation, amount of proteins and stearoyl-CoA concentration, this activity was always 2–3 times higher in the Trembler membrane fraction than in the normal one.

As a consequence, the level of available stearoyl-CoA for VLCFA biosynthesis is considerably lowered in the mutant.

Interestingly, the addition of ATP–Mg^{2+} always led to a decreased rate of stearoyl-CoA hydrolysis in the membrane fractions from Trembler and control mice. Thus, at 5 mM ATP–Mg^{2+} concentration, the levels of free fatty acid released from stearoyl-CoA were nearly the same in the two experimental systems.

Thus, one might ask whether that increase in stearoyl-CoA hydrolysis is related to the observed decrease in VLCFA formation by the membrane fraction from Trembler sciatic nerves [5].

A further study of the stearoyl-CoA elongation, in experimental conditions giving the same level of stearoyl-CoA hydrolysis in membrane fractions from normal and Trembler sciatic nerves, will answer this above question.

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