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A COMPARISON OF FATTY ACID ACTIVATION IN SCIATIC NERVE AND SCHWANN CELL RICH CULTURES FROM NORMAL AND TREMBLER MICE

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The activity of the fatty acid activating enzyme, palmitoyl-CoA synthetase, was measured in whole homogenates of sciatic nerve, cultured Schwann cells and fibroblasts, from normal and Trembler mice. A decrease in enzyme activity of 70% was measured in Trembler nerves and 35% in cultured Schwann cells from the Trembler mouse.

Myelination of peripheral nerve can be considered a simple model for the complex processes occurring in the central nervous system. The Trembler mouse [7] is a neurological mutant with abnormal myelin only in the peripheral nervous system (PNS) [3]. The Trembler lesion, which is primarily due to a Schwann cell defect [1, 2] which may involve the cell membrane, gives rise to pathological changes similar to those seen in human hypertrophic neuropathies.

Studies on the lipid composition of Trembler nerve show a 66% decrease in total lipid, together with a large decrease in cholesterol content [8]. Alkanes, an indication of myelination, are also reduced in the PNS of this mutant [6].

Cultured cell lines reflect some of the properties of the original tissue and thus may facilitate the understanding of complex processes. Bourre et al. [4], using cultured Schwann cells from normal and Trembler mice, were able to demonstrate changes in lipid metabolism which are consistent with those seen in the lipid composition of whole nerve.

The present study was designed to extend the above findings by measuring the activity of the fatty acid activating enzyme, palmitoyl-CoA synthetase EC 6.2.1.3, in homogenates of sciatic nerve and Schwann cell cultures from normal and Trembler mice.

Trembler and control mice were from the B6-CBA strain originally obtained from the Institute of Animal Genetics, Edinburgh, U.K. Following cervical dislocation, sciatic nerves (80–100 mg) between the spinal origin and the knee were removed, carefully freed of surrounding connective tissue and chilled on ice. Adult littermates were used throughout. Fresh weight was determined and the nerves were homogenized in ice-cold 0.32 M sucrose, pH 7.4 (1:10 w/v), using a close fitting, tapered, all glass homogenizer. The resultant homogenate was used immediately in the palmitoyl-CoA synthetase assay.

Peripheral nerve cell cultures were prepared as described by Bourre et al. [4] and Hauw et al. (unpublished). Cells from the 2nd, 3rd and 4th subcultures were used for the studies when confluency was reached for both Trembler and control. Cells were washed twice with PBS in the culture flasks. After scraping with a rubber policeman, a cell pellet was obtained by centrifuging for 5 min at 200 *g* (Rotor H54 in a Sorvall RC3). The pelleted cells were resuspended in ice-cold 0.32 M sucrose (pH 7.4) and homogenized in a close fitting, tapered, all glass homogenizer to ensure complete cell disruption. This was checked by light microscopy. The resultant homogenate was used directly in the palmitoyl-CoA synthetase assay.

Palmitoyl-CoA synthetase was assayed using the exchange reaction [8] and was a modification of that previously described [5]. Maximum synthetase activity (measured as palmitoyl-[^{3}H]carnitine) was obtained under the following conditions in duplicate incubations (5–10 min at 30°C): 100 mM Tris-HCl, pH 7.5, 80 μM CoA, 3.0 mM dithiothreitol, 1.5 mM ATP, 0.4 mM potassium palmitate, 3 mg albumin (fat free), 2 mM $MgCl_2$, 5 mM DL-[^{3}H]carnitine (0.5 Ci/mol), excess carnitine acyltransferase and homogenate in a total volume of 1 ml. Partially purified carnitine acyltransferase was prepared from calf liver mitochondria [8]. Carnitine acyltransferase and potassium palmitate were suspended in 1% Nonidet-P40 (Shell Chemicals). Reactions were terminated by the addition of 100 μl of 5 M HCl and then palmitoyl-[^{3}H]carnitine was extracted with 2 ml *n*-butanol saturated with distilled water.

Washed samples (200 μl) of the butanol phase were counted directly in scintillation fluid (NEN Aquasol) in a Packard 460 liquid scintillation spectrometer with automatic quench correction. In the determination of enzyme activity, linearity of incorporation of radioactivity into palmitoyl-[^{3}H]carnitine with respect to added homogenate and time was established. Protein concentration was determined by the method of Lowry et al. [10].

In homogenates of sciatic nerve, the activity of palmitoyl-CoA synthetase was reduced by 70% in the Trembler mutant. Control activity was 0.93 ± 0.03 nmol palmitoyl carnitine produced per mg homogenate protein per minute, whereas mutant activity was 0.29 ± 0.04 nmol/mg protein per minute. Since the protein content of peripheral nerve varies only slightly between adult normal and mutant mice (0.14 ± 0.02 and 0.11 ± 0.03 mg/mg fresh nerve, respectively) there is a large reduction in the activity of palmitoyl-CoA synthetase activity. Maximum enzyme

TABLE I

THE ACTIVITY OF ACYL-CoA SYNTHETASE IN SCHWANN CELLS AND FIBROBLASTS CULTURED FROM NORMAL AND TREMBLER MUTANT MICE

All values are the mean of 4 experiments. All experiments were carried out as detailed above. Time courses were used to determine the values. *$P<0.05$; **$P<0.1$.

	Activity (nmol palmitoyl carnitine/ mg protein/min)	% Control
Normal		
Schwann cell	0.17 ± 0.02	100
Fibroblast	0.34 ± 0.04	100
Trembler		
Schwann cell	0.11 ± 0.01*	65
Fibroblast	0.50 ± 0.06**	147

activity of the mutant animal was expressed under the same conditions as developed for normal nerve tissue.

In all cultures containing either Schwann cells or fibroblasts predominantly, the activity of palmitoyl-CoA synthetase was also altered. The cultures containing Schwann cells from Trembler mice showed only 65% of the palmitoyl-CoA synthetase activity measured in the control cultures (Table I). In parallel cultures containing fibroblasts there was an increase in synthetase activity under the same conditions. The reason for this increase in activity in fibroblast cultures from the Trembler mutant is unknown; however it has been noted several times before in similar experiments (J.M. Bourre and M. Monge, personal communications). The smaller difference in the activity of palmitoyl-CoA synthetase between cultures containing Schwann cells as compared with sciatic nerve homogenates may be due to a loss of sensitivity of the cells in culture since axonal contact is required to regulate the process of myelination [12]. The residual palmitoyl-CoA synthetase activity was measured optimally under the same conditions as used for control experiments which, therefore, may reflect only a change in the V_{max} of the enzyme.

Therefore, changes in lipid metabolism in Schwann cells cultured from the Trembler mutant [4], can in part be related to a decrease in the activity of palmitoyl-CoA synthetase. The importance of palmitoyl-CoA synthetase in the incorporation of endogenous and exogenous fatty acids into complex lipids cannot be overlooked, and thus a reduction in its activity may cause a reduced rate of lipid synthesis.

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