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Alteration of Sulfatide Synthesis in Control and Trembler Mice During Wallerian Degeneration and Remyelination

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ABSTRACT

Sulfatide synthesis from sulfate is much greater in the peripheral nerves of the Trembler mouse. After nerve transection, during Wallerian degeneration, this synthesis rate drops down very rapidly in both normal and Trembler mice. Twenty-four hours after permanent transection, the rate of synthesis is reduced by 80% in the mutant and 50% in the normal mouse. Four days after transection, the synthesis rate in the Trembler is only 9% of that observed in intact nerves, and 21% of that in the intact nerves of normal animals. After 5 d the synthesis remains constant. Thus, enhanced synthesis of sulfatides in the Trembler mouse is probably not caused by Wallerian degeneration.

After crush of the sciatic nerve, the synthesis rate decreases very rapidly in the normal mouse as it does after permanent transection. But during regeneration, from the 7th day, it rises dramatically and 14 d after crush, a 2.5-fold increase in the synthesis rate is observed, compared to that in the contralateral control nerve. This synthesis rate returns to normal 1 mo after crush. In the Trembler, the synthesis

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decreases for 2 d after crush and increases from then on, eventually reaching the value of the contralateral control Trembler nerve within 2 mo. In the mutant there is no prominent peak of sulfatide synthesis during regeneration.

Index Entries: Myelin, in peripheral nerve during Wallerian degeneration; peripheral nerve, sulfatide synthesis and; sulfatide synthesis, in peripheral nerve during Wallerian degeneration and remyelination; Trembler mouse, Wallerian degeneration of peripheral nerve in; regeneration of peripheral nerve and sulfatide synthesis rate; degeneration, of peripheral nerve and sulfatide synthesis rate; Wallerian degeneration, of peripheral nerve and sulfatide synthesis rate.

INTRODUCTION

First described by Falconer (1951), the Trembler mouse is characterized by a severe hypomyelination of the peripheral nervous system with segmental demyelination (Ayers and Anderson 1973, 1975, 1976; Low 1976a,b, 1977). The peripheral nervous system presents abnormal myelin with uncompacted sheaths proliferation of connective tissue, and onion bulb formations. In contrast, histological examinations of the brain do not reveal any lesions (Ayers and Anderson, 1973), although some hypermyelination is found (Bourre et al., 1980). The mutation results from a primary disorder of Schwann cells (Aguayo et al., 1977) with an abnormal persistence of postnatal Schwann cell proliferation (Perkins et al., 1981). Modifications of motor muscle innervation have been described with alterations of the histoenzymological pattern (Koenig et al., 1980). Biochemical abnormalities of the sarcoplasmic reticulum have been found (Averet et al., 1980). During hypomyelination a decrease in the lipid content of the peripheral nervous system (Larrouquere et al., 1978) including alkanes (Darriet et al., 1979) and alterations of physicochemical parameters as measured by spin-label studies (Viret et al., 1979) have been observed.

The synthesis of very long chain fatty acids is reduced in the peripheral nerve (Cassagne et al., 1980), possibly because of a drastic increase of acyl-CoA hydrolase (Boiron et al., 1982), and to a reduction of acyl-CoA synthesis found in both whole nerve and Schwann cells in culture (Cantrill et al., 1982). During postnatal development, Na^+K^+-ATPase and CNPase (2′,3′-cyclic nucleotide 3′-phosphodiesterase) decrease whereas 5′-nucleotidase increases with age (Bourre et al., 1982). Totally unexpected is the large increase in sulfatide synthesis in the whole nerve (Matthieu et al., 1980, 1981) as well as in Schwann cells in culture (Bourre et al., 1981).

The Trembler mutant presents some aspects that are characteristic of Wallerian degeneration, as shown for instance by the presence of choles-

terol esters (Larrouquere et al., 1978), and these molecules are considered to be possible markers of Wallerian degeneration (Yao et al., 1980). The present work was undertaken to determine whether the enhanced rate of sulfatide synthesis in the mutant was the result of Wallerian degeneration or was mutation-specific.

MATERIAL AND METHODS

All the animals were bred in our laboratory. Trembler mice (*Tr*/+) and their controls (+/+) were obtained by making *Tr*/ + heterozygotes with $B_6D_2F_1$ wild mice strain. Sixty-day-old animals were anesthetized with imalgene G-200 (Mérieux, France) injected intraperitoneally and permanent transection was performed as follows: depilation of the dorsal side of the left leg and hip was done, followed by alcohol cleansing of the skin. Under aseptic conditions the left sciatic nerve in normal and Trembler animals was exposed by separating the two muscles (through the connective tissue, epimysium) and the nerve was cut at the level of the proximal one-third of the femur. To avoid axonal regeneration from the proximal end, the distal stump was either attached to the skin (for long-term analysis) or simply turned back to the extremity of the leg (for short-term experiments). The wound was closed by one or two stitches. Standard unilateral crush injury was performed by applications of fine smooth forceps to the left sciatic nerve for 5 s. The right nerve was used as a control in both normal and Trembler mice. The weights of the freshly dissected nerves were measured. Nerves were incubated for 2 h in the presence of $H_2{}^{35}SO_4$ (40 μCi, the stock solution from NEN being neutralized with 10 m*M* phosphate buffer, pH 7.2). The Eagle MEM medium contained glutamine and was supplemented with 10% fetal calf serum; 0.5 mL were used for each nerve. The flasks were bubbled for a few seconds with oxygen–carbon dioxide (95/5, v/v). After incubation, the nerves were washed twice with the MEM Eagle medium, and were eventually dried with paper filter, lyophilized, and weighed. Tissues were homogenized with 5 mL chloroform–methanol (2/1, v/v) in a glass–glass tissue grinder, sonicated three times for 15 s, and stirred at room temperature for 1 h. After a Folch partition (Folch et al., 1957; Pollet et al., 1978) the lipids were separated by thin layer chromatography with chloroform–methanol–water (70/30/4, v/v/v) as the migrating solvent. Autoradiography was eventually performed; the spots were visualized by iodine, scraped, and counted using scintillation fluid. Values were expressed as cpm in sulfatides/mg dry weight of sciatic nerve. Incorporation of sulfate was linear up to 2 h in the control and Trembler mice after crush or transection. One point is the mean value of four experiments, each using one animal. We used 85 controls and 86 Trembler animals for this study.

RESULTS AND DISCUSSION

Figure 1 shows that sulfatide synthesis from sulfate is largely enhanced in the peripheral nerve of the Trembler, in agreement with results previously published (Matthieu et al., 1980, 1981). After nerve transection, the synthesis of sulfatides drops very rapidly in both normal and Trembler mice. Twenty-four days after permanent transection, the synthesis of sulfatides is reduced by approximately 80% in the mutant and 50% in the normal mice. Four days after transection, the synthesis of sulfatides in the Trembler mouse is only 9% of that observed in the intact right nerve of controls, and 21% of that observed in the intact right nerve of normal animals. The rate of synthesis is higher in Trembler Schwann cells undergoing Wallerian degeneration than in normal cells, in agreement with results obtained with Schwann cells in culture (Bourre et al., 1981).

The rapid decrease in sulfatide synthesis is probably also found in the central nervous system (in optic nerve), since Reigner et al. (1981) found a 40% decrease in the synthesis of sulfatides 1 d after retinal ablation. A rapid decrease (within 1 d after transection) is also found in phosphatidylethanolamine synthesis (Koeppen et al., 1982). In contrast there is an increased synthesis of phosphatidylcholine, within 5 d after transection, during degeneration (Natarajan et al., 1982; Koeppen et al., 1982).

For 7 d after crush of the sciatic nerve (Fig. 2), the synthesis drops very rapidly in the normal mouse, as it does in permanent transection. But during regeneration, from the 7th day, it rises dramatically, and 14 d after crush, a 2.5-fold increase in synthesis in the contralateral control nerve is observed. The synthesis returns to normal 1 mo after crush. In the Trembler, the rate of synthesis drops for 2 d after crush, and increases from then on, to reach the value of the contralateral control Trembler nerve within 2 mo. In the mutant there is no prominent peak in the rate of sulfatide synthesis during regeneration.

Interestingly, in the mutant, sulfatide synthesis decreases more rapidly after transection of the nerve than it does in control animals. This could be explained by the fact that sulfate turnover (the radiolabeled precursor used in this study) was enhanced in the Trembler (Matthieu et al., 1981). In the mutant, the sulfatide synthesis does not correlate with sulfatide deposition in myelin; the accumulation of sulfatides is prevented by an increased aryl-sulfatase (Matthieu et al., 1980).

These results are in agreement with those of Mirsky et al. (1980) showing that Schwann cells in culture stop making detectable amounts of sulfatides, in contrast with oligodendrocytes. In fact, Schwann cells in culture are able to synthesize minute amounts of sulfatides (Fryxell, 1980; Bourre et al., 1981), with the rate of synthesis being much lower than that in the whole nerve, where cells are myelinating.

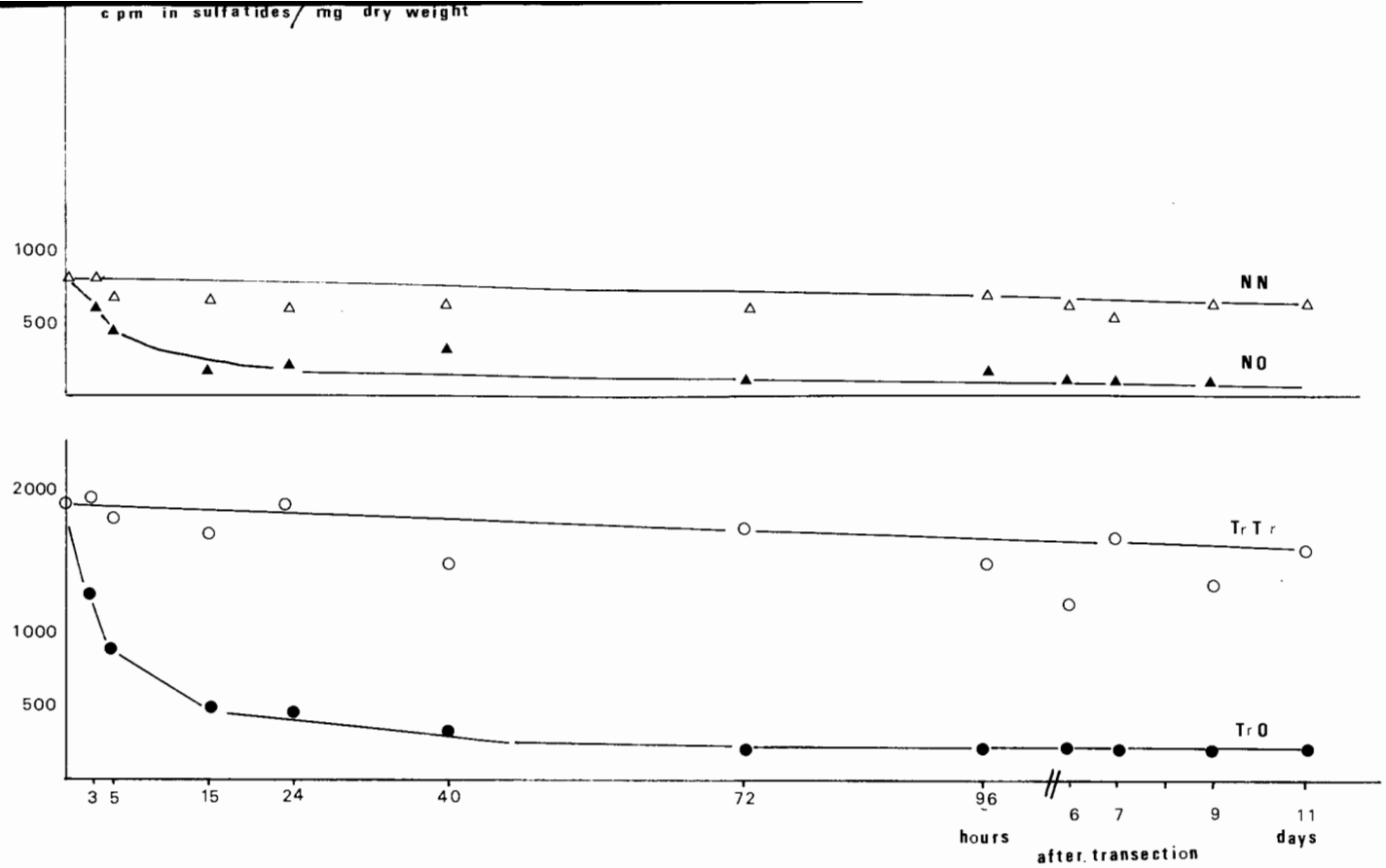

Fig. 1. Incorporation of sulfate into sulfatides-sciatic nerve in control and Trembler mice at various time after permanent transecton of the sciatic nerve: NN, control normal nerve without transection; NO, normal nerve with permanent transection; TrTr, trembler nerve without transection; TrO, trembler nerve with permanent transection. For both control and Trembler mice, sulfatide synthesis differs significantly between contralateral control nerve and transected nerve ($p < 0.01$ at least) except for 3 and 24 h in controls, and 3 h in Trembler mice. Note the break in the time scale.

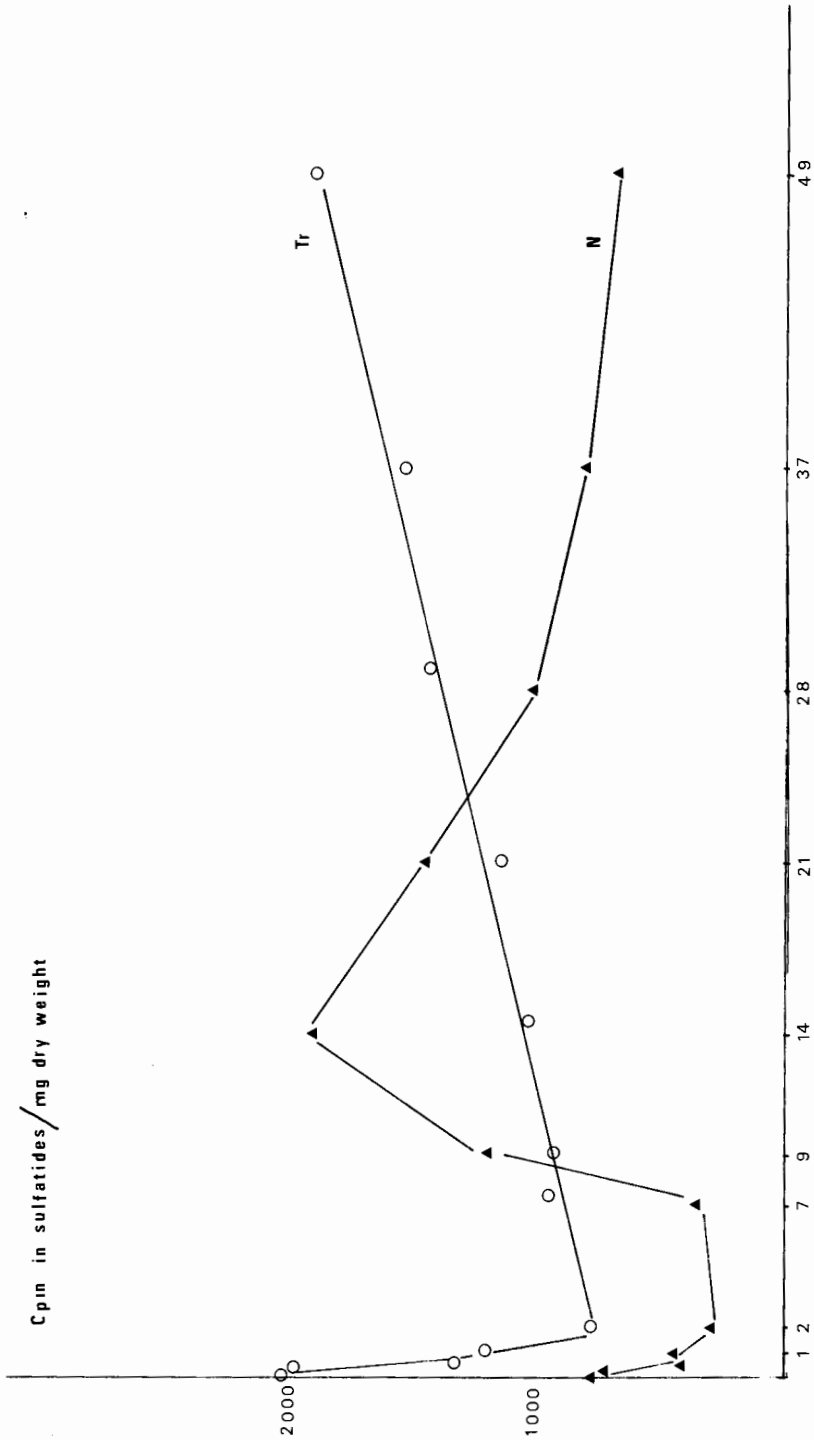

Fig. 2. Incorporation of sulfate into sulfatides in the sciatic nerve of control and Trembler mice at various times after crush of the sciatic nerve: N, normal animal; Tr, Trembler mutant. Tr differs significantly from normal ($p \leq 0.01$ at least) for all points except 9, and 21 d.

Thus the enhanced sulfatide synthesis in the Trembler, although possibly arising from Wallerian degeneration, is mutation-specific. Moreover, the Trembler Schwann cells can receive the axonal signal since during regeneration there is an increased sulfatides synthesis (Fig. 2).

The signal for dedifferentiation (arrest of producing sulfatides, a myelin lipid) and multiplication of Schwann cells is unlikely to come from myelin, but rather from the axolemma or from the axon (Salzer et al., 1980). The mitogenic signal for Schwann cells proliferation is located on the neurite surface, since Schwann cells require continuous input from myelin-inducing neurites for the production of myelin glycolipids (Mirsky et al., 1980). However, in the Trembler, the Schwann cells proliferate, although the axons are normal (Aguayo et al., 1977).

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