Journal of Neurochemistry Vol. 31, pp. 1541–1543
Pergamon Press Ltd. 1978. Printed in Great Britain

0022-3042/78/1201-1541$02.00/0

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SHORT COMMUNICATION

Sciatic nerve contains alkanes; comparison between normal mice and neurological mutants—Jimpy, Quaking and Trembler

(*Received* 17 *May* 1978 *Accepted* 3 *July* 1978)

THE NEUROLOGICAL mutants Jimpy, Quaking, and Trembler are well known for their abnormal myelination involving peripheral nervous system (PNS), and/or central nervous system (CNS).

Under light (SIDMAN *et al.*, 1964; SIDMAN & HAYES, 1965) and electron microscopy (HERSCHKOWITZ *et al.*, 1971) the PNS of the Jimpy mouse is supposed to be normal. Although apparently normal under light microscopy (SIDMAN *et al.*, 1964) the peripheral nerves of Quaking present under electron microscopy several unusual features in the pattern of myelination in addition to hypomyelination (SAMORAJSKI *et al.*, 1970; SUZUKI & ZAGOREN, 1976, 1977). Schwann cell transplantation has shown some evidence for a primary sheath cell disorder causing hypomyelination in these Quaking mice (AGUAYO *et al.*, 1977*b*).

In contrast to Jimpy and Quaking mutants the main neurological abnormality of the Trembler affects the PNS but the brain is apparently normal (AYERS & ANDERSON, 1973).

One major aspect of this mutant is the development of onion bulb formations (AYERS & ANDERSON, 1975; LOW & MCLEOD, 1975; LOW, 1976*a*, *b*, 1977). The Trembler mouse is considered as a model for human Dejerine–Sottas disease (AYERS & ANDERSON, 1973). AGUAYO *et al.* (1977*a*), demonstrated recently that the neuropathy in the Trembler mouse is due to a primary defect of Schwann cells. As far as we know the Trembler mouse has not been biochemically investigated.

Whereas the occurrence of alkanes, in whole brain, was known (NICHOLAS & BOMBAUGH, 1965; DANNENBERG & RICHTER, 1968) we demonstrated that they accumulated specifically in the mouse brain myelin (DARRIET *et al.*, 1978); the level was reduced by two thirds in the Quaking mutant (BOURRE *et al.*, 1977*a*), in which the oligodendrocyte is probably defective. The purpose of this work is to study the presence of these molecules in PNS of normal mice and neurological mutants, to test whether the alkane amount and its distribution pattern could reflect an altered myelination.

MATERIALS AND METHODS

Sciatic nerves were carefully removed from neurological mutants and control mice of the same age and similar body weight. Young mice 18-day-old Quaking and Jimpy (26 mice of each) and adult Quaking and Trembler mice (35 Quaking and 16 Trembler) were used.

We obtained the Quaking mutant on C57-B6 strain, both Jimpy and Trembler were on B6-CBA strain. The weights of fresh and lyophilized nerve were determined. Both young and adult mice were used as Jimpy mice do not reach adulthood.

Alkane analysis: the solvents used in this study were freshly redistilled ($CHCl_3$, MeOH, spectrograde hexane). All the glassware was carefully washed, boiled with redistilled methanol and washed with spectrograde hexane. In each case a complete blank run was done, including all the reagents and solvents without sample, in order to detect any laboratory contamination. Alkanes were never detected in these conditions, and corrections for contamination were therefore unnecessary.

Dry material (2–8 mg) or fresh material (2–40 mg) plus octadecane (80 μg) as internal standard were saponified with 5 M-KOH (0.5 ml) at 70°C for 1 h. The nonsaponifiable matter, including alkanes was extracted thrice with 2 ml spectrograde hexane. The hexane solution was washed with water, dried with Na_2SO_4 and concentrated to 1 ml. This solution was applied to a glass column (30 cm × 3 mm), containing alumina (activity degree III), which had been prewashed with 30 ml of spectrograde hexane and eluted with 30 ml of spectrograde hexane. The eluate was evaporated to 100 μl under N_2 and the alkane solution was kept frozen, for GLC analyses.

The analyses of the alkanes were done by GLC on a 10% SE 30 column. The alkane quantification was performed with a computer (IPAC 10 from LTT, Paris); the quantities were also estimated by calculation of the peak areas, as compared to that of the internal standard, assuming a similar response of the detector for all the alkanes. Each experiment was performed at least 3 times.

RESULTS AND DISCUSSION

Table 1 shows that the dry weight of the sciatic nerve is near 30–35% of the fresh weight in the normal, Quaking and Jimpy mice, but only about 20% for the Trembler

TABLE 1. CONTENT OF ALKANE IN FRESH AND DRY SCIATIC NERVE OF NORMAL AND NEUROLOGICAL MUTANTS (QUAKING, JIMPY AND TREMBLER)

	Solids sciatic nerve dry weight/ fresh weight (%)	Alkanes fresh sciatic nerve (μg/mg)	Alkanes dry sciatic nerve (μg/mg)
Control	33.2	1.12 (8)	3.25 (4)
Trembler	20.5	0.34 (7)	1.56 (4)
Quaking	31.6	0.47 (4)	1.37 (4)
Jimpy	32	0.72 (6)	2.64 (2)

Control: control littermate; Quaking and Trembler are adult animals, Jimpy are 18-day-old; number in brackets represents the number of experiments; the first column shows the dry/fresh sciatic nerve yield expressed as a percentage.

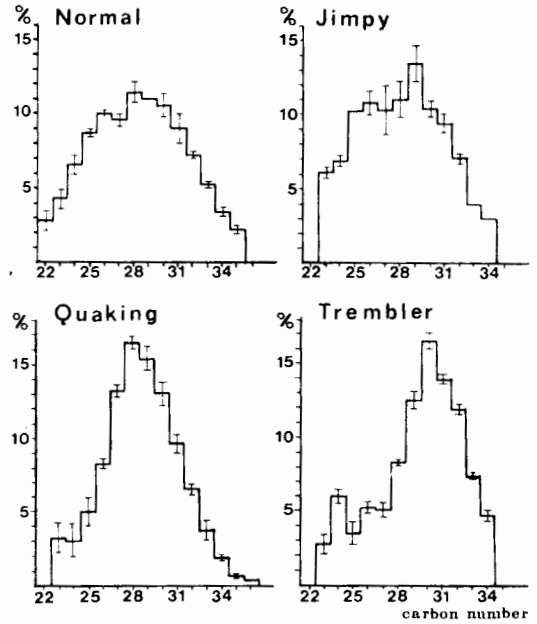

FIG. 1. Alkane distribution pattern from the sciatic nerves of normal mouse and neurological mutants (Jimpy, Quaking and Trembler). Results are given as the % of each alkane according to their chain length.

one. This striking difference, observed repeatedly in the Trembler sciatic nerve, not yet explained, but possibly due to the severe hypomyelination, led us to perform the analyses, in the same manner, on fresh and lyophilized sciatic nerves.

No significant difference in the quantity of alkanes found in the sciatic nerves from adults and from 18-day normal mice have been noted. This is in good agreement with the fact that PNS myelination is achieved around that age (RAINE, 1977).

When we consider the results (Table 1) obtained from fresh sciatic nerves, every studied mutant exhibits smaller alkane content than the control. This alkane content is largely reduced in the Trembler PNS (66%) whereas the diminution is about 50% in the Quaking and 30% in the Jimpy mutants.

On the other hand, when the alkanes are obtained from lyophilized sciatic nerve the reduction of their level in the Jimpy mutant represents 20%, but is between 40 and 50%, for the Quaking and Trembler mutants. The difference in the reduction of the alkane amount found in fresh and dry sciatic nerve of mutants, is explained by the difference of the dry/fresh yield.

Figure 1 shows the alkane distribution pattern from the sciatic nerves of the neurological mutants compared to the normal one.

The normal control littermate from the three mutants, particularly from the adults and 18-day mice, presents the same biochemical composition.

Every alkane, from C22 to C35 is present in normal sciatic nerve, from C23 to C34 in the Jimpy mutant, from C23 to C36 in the Quaking mutant and from C24 to C34 in the Trembler. In each case, there is an almost equal distribution between odd and even chains.

Alkanes from Quaking and Trembler sciatic nerve have nearly the same profile which differs from the normal one, in that long chain alkanes (C28, C29, C30 for Quaking and C29, C30, C31 for Trembler) are significantly increased whereas shorter chains (C23, C24, C25 for Quaking and C25, C26, C27 for Trembler) are markedly reduced. In the Jimpy mouse, the alkane profile appears normal.

From biochemical and morphological data (HERSCHKOWITZ *et al.*, 1971) the sciatic nerve of the Jimpy mouse appears normal: from our results, there is only a small decrease in alkane content which exhibits however a normal distribution pattern.

Both reduction of the alkane level and abnormal distribution pattern of these remaining alkanes occur in Quaking and Trembler sciatic nerves, whose PNS alterations, due to a primary Schwann cell defect, have been reported above. Moreover the highest reduction is encountered in Trembler sciatic nerve which presents the most altered structural aspects.

Trembler mouse, as the model of a human inherited neuropathy, would be also a good model for studying peripheral hypomyelination.

In conclusion, alkanes are found in mouse PNS, where their level and composition seem to be related to the degree of myelination of the sciatic nerve.

Only fragmentary data are available on alkane formation. We reported that a microsomal pellet from rabbit sciatic nerve is able to synthesize both very long chain saturated fatty acids and alkanes from stearic acid with the same cofactors (CASSAGNE *et al.*, 1977, 1978). In addition, very long chain fatty acids and alkanes are reduced (in the same order of magnitude) in the Quaking brain myelin as compared to the normal one (BOURRE *et al.*, 1977*a*), so that alkanes could originate from very long chain fatty acids, which are synthesized in both central (BOURRE *et al.*, 1977*b*) and peripheral nervous system (CASSAGNE *et al.*, 1978) by a reductive decarboxylation process. Work is now under progress in our laboratory to compare the alkane biosynthesis in normal mouse and neurological mutant.

Acknowledgements—The authors are grateful to F. LACHAPELLE for breeding Quaking and Jimpy mice.

They thank Professor J. L. GUENET, Institut Pasteur, Paris, for providing Trembler animals.

This study was supported by grants from INSERM and CNRS.

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