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Ketone Body Utilization for Lipid Synthesis in the Murine Sciatic Nerve: Alterations in the Dysmyelinating Trembler Mutant

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Abstract: This work demonstrates that in vitro sciatic nerves of normal and trembler adult mice can use ketone bodies (β-hydroxybutyrate and acetoacetate) and butyrate for lipid synthesis. In normal sciatic nerves, β-hydroxybutyrate is incorporated in total lipids to a larger extent than acetoacetate (141% and 33%, respectively, of acetate incorporation), whereas for trembler sciatic nerves, these percentages are only 69% and 27%. Incorporation of ketone bodies is greater into sterols than into other lipids. Lipid metabolism of ketone bodies in trembler nerves is altered and could reflect a process similar to Wallerian degeneration: a dramatic decrease of sterol and free fatty acid synthesis and an increased synthesis of triglycerides. Moreover, differences seen in precursor incorporation into lipids between normal and trembler sciatic nerves suggest that their lipid metabolism is not the same. **Key Words:** Ketone bodies—Lipids—PNS—Trembler. **Clouet P. M. and Bourre J.-M.** Ketone body utilization for lipid synthesis in the murine sciatic nerve: Alterations in the dysmyelinating trembler mutant. *J. Neurochem.* **50**, 1494–1497 (1988).

Synthesis and maintenance of a functional myelin sheath are dependent on lipid metabolism by the myelinating cells. In general, in the brain, glucose is the most important precursor for these processes. It has been demonstrated, however, that ketone bodies (acetoacetate and β-hydroxybutyrate) can be used as precursors for lipid synthesis in developing brain (Page et al., 1971; Patel and Owen, 1977; Yeh et al., 1977) and during prolonged starvation (Owen et al., 1967; Edmond et al., 1985; reviewed by Robinson and Williamson, 1980). Whether or not butyrate can be used for lipid synthesis is unknown. Studies both in vitro and in vivo have shown that ketone bodies are used preferentially by the developing brain for the synthesis of lipids, especially cholesterol (Webber and Edmond, 1979; Yeh, 1980; Koper et al., 1981). Similar results were obtained for oligodendrocytes in culture (Pleasure et al., 1979; Koper et al., 1984; Sykes et al., 1986) but not in astrocytes or neurons in culture (Lopes-Cardozo et al., 1986). Nothing is known, however, about the utilization of ketone bodies in the PNS. We therefore have investigated their use in sciatic nerves from normal and dysmyelinating trembler mice. The mutant is characterized by a hypomyelinating peripheral neuropathy, presumed to be due to a primary metabolic disorder of the Schwann cell (reviewed by Bourre et al., 1984). This results in impairment of differentiation and increased proliferation of Schwann cells. The quantities of all lipids, except cholesterol esters, are reduced (Larrouquère-Regnier et al., 1979).

In studying incorporation of ^{14}C-labelled acetate, butyrate, and ketone bodies into lipids of normal and trembler adult mouse PNS, we sought to determine (1) if the normal PNS uses these compounds in lipid synthesis and (2) if perturbations of Schwann cell differentiation in trembler mice induce changes in the utilization of these precursors in lipid metabolism.

MATERIALS AND METHODS

Solvents, salts, and silica gel plates (60F254) were from Merck (FRG). [1-^{14}C]Acetic acid, sodium salt (sp act. 55 mCi/mmol) and [1-^{14}C]butyric acid, sodium salt (sp act. 49 mCi/mmol) were from CEA, France; [3-^{14}C]β-hydroxybutyric acid, potassium salt (sp act. 48.4 mCi/mmol) was from New England Nuclear. Ethyl [3-^{14}C]acetoacetate (sp act. 6.6 mCi/mmol) was from Amersham, France. [3-^{14}C]-Acetoacetate was prepared by alkaline hydrolysis of ethyl [3-^{14}C]acetoacetate in 0.1 *M* KOH at 37°C for 1 h followed

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neutralization with 0.1 M HCl. Liquid scintillation fluid was Ready-Sol HP from Beckman.

Sciatic nerves from 60-day-old normal and trembler mice were carefully removed and desheathed on a cold plate. Each incubation contained four nerves in 1 ml of oxygenated Krebs' glucose medium, pH 7.4 (described by Dupuis and Towfighi, 1972). Preparations were incubated at 37°C for 2 h with either 20 μCi of [1-^{14}C]acetate or [1-^{14}C]butyrate, or 10 μCi of [3-^{14}C]hydroxybutyrate or [3-^{14}C]acetoacetate. After incubation, samples were put on ice and washed twice with 1 ml of modified Krebs' medium. The lipids were extracted by the method of Yao and Cance (1983). An aliquot of the lipid phase was used for measuring the total incorporation of precursors into nerve lipids. The separation of neutral lipids was performed by TLC using hexane/diethyl ether/acetic acid (75:23:2, by vol) as developing solvent. Phospholipids and glycolipids were separated according to Vitellio and Zanetta (1978). Lipids were visualized by exposure to iodine vapor and radioactivity was measured after scraping the spots directly into scintillation fluid. Protein content was determined according to Lowry et al. (1951). Results were standardized for a theorical solution of 1 m*M* in the incubation medium.

RESULTS

Table 1 shows that the incorporation of all precursors was greater in normal sciatic nerves than in trembler nerves. In normal sciatic nerves, precursors were incorporated in the following rank order: β-hydroxybutyrate > acetate > butyrate > acetoacetate, with four times more hydroxybutyrate incorporated than acetoacetate. But in trembler mice sciatic nerves, the level of incorporation of precursors was different: acetate, butyrate, and hydroxybutyrate were almost equally incorporated, whereas the level of acetoacetate incorporation was half this value. The percentage distribution of radioactivity in different lipid classes shows that in normal sciatic nerves, the neutral lipids were more labelled than the phospholipids, especially with ketone bodies producing increased cholesterol esters. The sterols were the most labelled class of lipids and the free fatty acids relatively were more labelled with butyrate. Labelled phospholipids were relatively synthesized more from acetate (mainly phosphatidylcholine and phosphatidylethanolamine). On the other hand, cerebrosides were relatively labelled less with hydroxybutyrate than with other precursors. In the trembler sciatic nerves, neutral lipids were not the most labelled lipids, and the relative percentage of labelled sterols was lower than in normal sciatic nerve (this decrease was more significant with acetate and butyrate). Free fatty acid levels were lower. In contrast, triglycerides were labelled in trembler more than in normal mouse sciatic nerves. In trembler mice, acetoacetate incorporation in triglycerides was greater than that of acetate. In trembler mice, all phospholipids were labelled

TABLE 1. *Incorporation of ^{14}C-labelled precursors into lipids in sciatic nerves from normal (N) and trembler (Tr) mice*

	Acetate		Butyrate		β-Hydroxybutyrate		Acetoacetate	
	N	TR	N	TR	N	TR	N	TR
Total incorporation into lipids (nmol/mg protein/h)	2.7 ± 1.0	1.5 ± 0.9^{j}	1.6 ± 0.4^{b}	1.1 ± 0.3	3.8 ± 1.0^{e,h}	1.04 ± 0.5^{e,j}	0.9 ± 0.6^{b,h}	0.43 ± 0.2^{c,h}
Incorporation into each lipid class (% of total labelled lipids)								
Phospholipids								
PC	24.5 ± 3.3	35.1 ± 3.4^{k}	17.8 ± 1.9	36.7 ± 2.5^{k}	15.1 ± 2.6^{e}	21.9 ± 2.9^{e,e}	15.5 ± 1.8^{e}	27.5 ± 2.2^{b,k}
PS	2.1 ± 0.4	3.2 ± 0.9^{j}	1.9 ± 0.2	3.1 ± 0.7^{j}	1.8 ± 0.2	2.9 ± 0.3^{j}	2.0 ± 0.4	2.8 ± 0.2^{j}
PI + PA	2.5 ± 0.5	3.3 ± 0.8^{j}	2.0 ± 0.3^{c}	3.4 ± 0.3^{k}	1.9 ± 0.5^{a}	2.2 ± 0.2^{b}	1.6 ± 0.6^{c}	2.2 ± 0.3^{a}
PE	4.3 ± 0.8	4.8 ± 0.3	3.0 ± 0.5^{c}	4.8 ± 0.2^{k}	2.3 ± 0.4^{c,d}	3.5 ± 0.7^{b,d}	3.3 ± 0.8^{c}	4.1 ± 0.1
Glycolipids								
LPC + SPM	2.5 ± 1.4	3.8 ± 0.9^{j}	1.8 ± 0.6	3.1 ± 0.3^{j}	1.5 ± 0.6	3.6 ± 1.2^{j}	1.4 ± 0.4	3.6 ± 0.1^{k}
S	0.4 ± 0.2	0.7 ± 0.2^{k}	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.02^{b}	1.1 ± 0.4^{a,k}	0.2 ± 0.2^{b}	0.7 ± 0.1^{k}
CER	4.6 ± 0.8	7.1 ± 1.5^{j}	4.3 ± 1.4	6.5 ± 2.3^{j}	2.5 ± 0.4^{c,e,h}	6.5 ± 1.2^{j}	4.1 ± 0.2	5.7 ± 0.9^{j}
Neutral lipids								
DG + MG	2.0 ± 1.5	1.2 ± 0.3	1.0 ± 0.4	1.1 ± 0.2	2.1 ± 0.2	3.1 ± 1.7	1.1 ± 0.2	2.1 ± 1.1
ST	35.8 ± 8.0	13.9 ± 2.3^{k}	38.9 ± 8.0	15.7 ± 2.3^{k}	31.6 ± 1.0	25.9 ± 6.1^{b,d,g}	33.6 ± 6.0	16.5 ± 5.9^{k}
FFA	9.0 ± 2.8	1.7 ± 0.4^{k}	14.2 ± 4.8^{b}	1.9 ± 0.5^{k}	8.8 ± 5.1^{h}	2.1 ± 1.3^{j}	10.8 ± 3.1^{h}	3.0 ± 1.1^{k}
TG	4.1 ± 2.2	11.6 ± 4.0^{j}	4.3 ± 2.5	13.4 ± 3.3^{k}	4.4 ± 0.8	9.8 ± 3.4^{j}	3.7 ± 1.9	15.5 ± 2.7^{k}
CE	3.6 ± 5.4	3.1 ± 1.2	5.2 ± 2.5	3.2 ± 0.3	22.6 ± 6.5^{c,d}	3.4 ± 1.7^{k}	12.5 ± 7.5^{a}	6.7 ± 3.0^{b}

CE, cholesterol esters; CER, cerebrosides; DG + MG, diglycerides + monoglycerides; FFA, free fatty acids; LPC + SPM, lysophosphatidylcholine + sphingomyelin; N, normal; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI + PA, phosphatidylinositol + phosphatidic acid; PS, phosphatidylserine; S, sulfatides; ST, sterols (cholesterol + lanosterol + demosterol); TG, triglycerides; TR, trembler.

^{a,b,c} Significant difference ($p < 0.5$, $p < 0.01$, $p < 0.001$, respectively) compared to acetate incorporation in the same mouse.

^{d,e,f} Significant difference ($p < 0.5$, $p < 0.01$, $p < 0.001$, respectively) between hydroxybutyrate and acetoacetate incorporations.

^{g,h} Significant difference ($p < 0.5$, $p < 0.01$, respectively) compared to butyrate incorporations.

^{j,k} Significant difference ($p < 0.5$, $p < 0.01$, $p < 0.001$, respectively) between incorporation of one precursor in normal mice and the same precursor in trembler mice.

by precursors more than in normal nerves. These increases were more marked with butyrate. Sulfatide levels were relatively higher with ketone bodies in trembler mouse sciatic nerves.

DISCUSSION

This work demonstrates for the first time that mouse sciatic nerves can use butyrate and ketone bodies for in vitro lipid synthesis. We have confirmed a lower acetate incorporation into lipids, an increase of labelled triglycerides and cerebrosides, and a decrease of labelled free fatty acids in trembler as compared to normal sciatic nerves (Bourre et al., 1981; Yao and Bourre, 1985). The lower synthesis of lipids in trembler nerves obtained with acetate (Larrouquère-Regnier et al., 1979) was also obtained with all precursors.

In the normal PNS, the level of precursor incorporation decreased in the following order: β-hydroxybutyrate (141%), acetate (100%), butyrate (59%), and acetoacetate (33%). Lopes-Cardozo and Klein (1984), studying the developing brain, found the following order: acetoacetate (100%), β-hydroxybutyrate (82%), and acetate (33%). In contrast, we found a considerable difference between the incorporation of β-hydroxybutyrate and acetoacetate, the latter being only weakly incorporated. Thus adult PNS and developing CNS do not use ketone bodies in the same manner. In trembler sciatic nerves, utilization was: acetate (100%), butyrate (73%), β-hydroxybutyrate (69%), and acetoacetate (27%). The most substantial decrease occurred with hydroxybutyrate. The two ketone bodies appeared to be used less in lipid synthesis than was acetate. Interestingly, the mutation did not affect the incorporation of acetoacetate and β-hydroxybutyrate equally.

Ketone bodies were appropriate precursors for sterol labelling, relative to the other lipids. This was also true with acetate and butyrate. In contrast to the results of Koper et al. (1981) with brain myelin, ketone bodies were incorporated more into phosphatidylcholine and less into cerebrosides, sulfatides, and phosphatidylethanolamine in mouse sciatic nerves. Interestingly, cholesterol esters were synthesized more from ketone bodies than from acetate and butyrate in controls, but not in trembler mice. Acetate and butyrate were equally incorporated into the various classes of lipids in trembler and phospholipids were synthesized less from ketone bodies. This study confirms that there is an unexplained increased synthesis of sulfatides from acetate, previously shown in whole nerves (Matthieu et al., 1980) and in Schwann cell cultures (Bourre et al., 1981), and shows that increased synthesis is also found with ketone bodies. Although sterols were less labelled with all precursors in trembler sciatic nerves, they were more labelled with ketone bodies than with acetate.

As seen with acetate in degenerating nerves (Yao and Cannon, 1983), the incorporation of ketone bodies in trembler sciatic nerves could reflect a process similar to Wallerian degeneration with dramatic depression of sterol synthesis, increase of labelled triglycerides, and decrease of labelled free fatty acids. The mutation does not affect ketone body and acetate utilization in the same way, however. The differences between the lipid profiles with acetate, butyrate, and ketone bodies suggest that these precursors are used in nonidentical ways for lipid synthesis.

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