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PERIPERAL NERVE CELLS IN CULTURE RICH IN SCHWANN CELLS INCORPORATE AND METABOLIZE *TRANS*-UNSATURATED FATTY ACID (ELAIDIC ACID) AS WELL AS PHYSIOLOGICAL *CIS* ISOMER (OLEIC ACID)

JEAN-MARIE BOURRE, JEANNE-MARIE BOUTRY*, MARTIAL MASSON and JEAN-JACQUES HAUW*

*Laboratoire de Neurochimie, INSERM U 134 and *Laboratoire Charles Foix, Hôpital de la Salpêtrière, 75651 Paris Cedex 13 (France)*

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A culture of peripheral nerve cells enriched in Schwann cells was obtained from sciatic nerve in normal and dysmyelinating trembler mutant. These cells incorporated and metabolized a non-physiological *trans* fatty acid (elaidic acid) as well as the physiological *cis* isomer (oleic acid). Both acids were incorporated similarly in all lipids studied (phosphatidylcholine was a very potent acceptor) only cholesterol-esters' formation was slightly reduced from elaidic acid. Both acids were partially degradated into sub-units, in turn used for synthesis of new fatty acids. However elaidic acid was less degraded by the cells thus providing more C14:1, C 16:1 fatty acids and less cholesterol. The sub-units were also used to provide very long chains, saturated and mono-unsaturated; only synthesis of nervonic acid was at variance when using oleic and elaidic acids. The presence of elaidic acid diminished the elongation-desaturation of essential fatty acids.

No major differences were found between control and trembler cells, however cholesterol-esters' synthesis was slightly enhanced in the mutant cells, when using both acids.

trans Unsaturated fatty acids are formed during partial hydrogenation of unsaturated fatty acids by some rumen microorganism or by commercial processing of vegetable oils. Thus *trans* unsaturated fatty isomers, geometric of the natural *cis* isomer, may be quantitatively found in the diet in significant amounts.

trans Fatty acids are found in very minute amount in tissues from mammals, including the brain [23]. *trans* Fatty acids are absorbed and incorporated into most tissues of experimental animal and human [3, 8, 18], but it has been stated that brain selectively excludes *trans* fatty acids [25]. Although the rate of fatty acid metabolism is lower in brain than in other tissues, and nervous tissue is more selective in utilization of circulating fatty acids [6, 11], elaidic acid injected intragastrically is incorporated and metabolized by the developing brain [10], corroborating the fact that brain is able to utilize intracerebrally injected elaidic acid [9, 19].

trans Fatty acids are prevalent components of natural and processed food

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(margarines and processed oils) and may constitute a significant portion of the human diet (in some countries the consumption of processed margarines has increased to surpass the consumption of butter [26] and these compounds contain 2–10 times more *trans* fatty acids). *trans* Fatty acids could possibly alter peripheral nervous system function, this hypothesis being supported by the fact that accumulation of phytanic acid in human Refsum disease induces dramatic neurological symptoms (this branched chain non-physiological fatty acid is not degraded due to an inborn error of metabolism).

This study was designed so as to determine whether peripheral nerve cells in culture (mostly Schwann cells) were able to incorporate and metabolize elaidic acid as well as oleic acid (*trans* and *cis* isomers, respectively). Cells from control were compared to cells originated from trembler, a dysmyelinating mutant, with onion bulb formation [2, 22] due to defective Schwann cells [1] and presenting abnormal sulfatide metabolism in the whole peripheral nervous system [27] as well as in peripheral nerve cells in culture [7].

Enriched Schwann cells culture from 12-day-old control and mutant mice were developed from sciatic nerves as previously described [7]. The third to the fifth subcultures were used. When confluency was reached, cells were cultivated for 17 h in the presence of albumin bound [10-^{14}C]fatty acid (0.1 μmol of oleic or elaidic acid per dish, 45 mCi/mmol) from CEA (France). Cells were washed 3 times (3 min, 1000 *g*) with 0.9% saline directly in the flasks, released with a rubber policeman and a pellet was obtained by centrifugation. The cells were further washed twice; the pellet was lyophilized and lipid extracted with chloroform–methanol 2:1 [13] and sonication [5, 30]. Phospholipids and sphingoglycolipids were separated as a whole by thin layer chromatography (to discard non-esterified fatty acids, cholesterol, cholesterol esters and glycerides) the solvent used was chloroform–acetic acid 90:10; the ratio cholesterol to cholesterol esters was also determined using hexane–ether–acetic acid 90:10:1 [17]. Individual phospholipids and sphingoglycolipids were further separated using chloroform–methanol–water as a migrating solvent (70:30:4) [32]. Lipids were visualized by iodine, the spots scraped and either counted by liquid scintillation 'ready solv.', Beckman, or extracted with 3 times 5 ml of chloroform–methanol 2:1. Lipids were methanolyzed and fatty acid methyl esters were separated by thin layer chromatography. After extraction with pentane, they were analyzed by gas–liquid chromatography column carbowax 20 M; on emergence from the column, labeled fatty acids were collected in anthracene and the radioactivity was determined. Thin layer chromatographies were performed on silicagel 60 F 254 (Merck). Four experiments were performed using 12 mutant mice and 12 controls. Level of significance was performed according to Student's *t*-test.

Cells were cultivated in the presence of the same amount of oleic acid or elaidic acid with the same specific radioactivity; as shown in Table I, both acids' incorporation was similar. The differences were not significant. Of the added acid, 20–30% was taken up by the cells.

TABLE 1

INCORPORATION OF OLEIC ACID AND ELAIDIC ACID IN THE LIPIDS OF NERVE CELL CULTURES ENRICHED IN SCHWANN CELLS.

Values are the means from 4 experiments (8 analyses). O = oleic acid; E = elaidic acid. *$P < 0.05$; others are not significantly different.

	Control cells			Trembler cells		
	O		E	O		E
10^{-3} cpm incorporated/mg lipid extract	718		918	921		1237
Lipids %						
Free fatty acids	7.5		5.1	5.6		3.9
Cholesterol	1.1	*	0.7	0.9	*	0.5
Cholesterol esters	6.3	*	4.9	13.2	*	9.2
Sphingomyelin	2.7		1.6	4.1		1.5
Choline phosphoglycerides	52.2		57.3	55.1	*	66.5
Inositol + serine phosphoglycerides	8.1		11.9	4.0		7.2
Ethanolamine phosphoglyceride	9.4		7.9	9.2		2.7
Miscellaneous	12.7		10.6	7.9		8.5

Most of the fatty acids taken up were incorporated either as such into lipids, or eventually after being metabolized (Table I); only a few percent of the radioactivity was found in non-esterified fatty acids. Labels from oleic and elaidic acids were incorporated in a similar way into different phospholipids, choline, phosphoglycerides being the more potent acceptor. Label from elaidic acid was significantly reduced in cholesterol and cholesterol esters as compared with label from oleic acid.

There was no major difference between control and mutant cells, except that cells from trembler produced increased amount of cholesterol ester in agreement with analysis of the sciatic nerve [20].

The fatty acid analysis in Table II showed that most of the label was found in the original acid. However this acid was partially degraded, producing shorter chains; eventually acetate units were formed and used in the synthesis of long and very long chain fatty acids. If compared to oleic acid, more C 16:1 and C 14:1 were produced from elaidic acid; on the contrary, acetate units from elaidic acid were less utilized in the elongation–desaturation processes of essential fatty acids.

Very similar profiles were obtained with cells from trembler mutant.

Elaidic acid was largely incorporated as such in cell lipids, in a similar way to oleic acid. However, this acid was apparently only partially degraded (thus explaining the accumulation of C 16:1 and C 14:1). It is apparently degraded at a slower rate as compared to oleic acid; thus, lesser amounts of acetate units were produced and less acetate units were utilized in chain lengthening of essential fatty acids. However, inhibition of elongation–desaturation processes of essential fatty acids was not excluded.

TABLE II

INCORPORATION OF THE RADIOACTIVITY FROM OLEIC ACID AND ELAIDIC ACID IN THE FATTY ACIDS OF NERVE CELL CULTURES ENRICHED IN SCHWANN CELLS.

Same legend as in Table I. $^{*}P < 0.05$; $^{**}P < 0.01$; others are not significant.

Chain length	Oleid acid %		Elaidic acid %
14:1	0.1	*	0.5
16:1	1.3	**	6.5
16:0	0.9		1.9
17	0.6		0.6
18:1	68.3		65.5
18:0	5.6		3.7
20:ϱ	4.6	**	2.4
20:1	1.2		1.3
20:0	0.9		1.1
22:ϱ	1.4	*	0.9
22:1	0.5		0.7
22:0	0.5		0.7
24:1	3.1	**	0.6
24:0	0.5		0.7

Alterations in the fatty acid composition cause significant changes in the physical properties of the membranes [24, 31]; in *E. coli*, the ability of cell membranes to transport sugar [21] and to exhibit functional Na, K-ATPase activity depends on membrane fluidity, when manipulating membrane fatty acids [34]. Changes in fatty acids affect the activity, hormone response and temperature dependence of adenylate cyclase [12]. Manipulation of fatty acid composition of mouse LM cells affects , membrane integrity and fluidity and disturbs cell growth [29].

The present results show that the non-physiological *trans* elaidic acid is incorporated by nerve cells in culture and eventually further metabolized. Since this acid can contribute to the constitution of the Schwann cells membrane, what effects might this have on cell function and capacity? As elaidic acid is incorporated in brain cells after being ingested by an animal, it is also probably incorporated in the nerve in vivo as our results show that Schwann cells incorporate this acid. Thus occurrence of elaidic acid in the peripheral nervous system could alter the function of nerves: few immediate toxic effects of *trans* fatty acids in the diet have been described [16] and some physiological and long-term pathological consequences have been implied [14, 15, 28, 33].

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