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Incorporation of Stearic Acid Into Brain Lipids in the Developing Brain: Blood-Brain Relationships during Development

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Abstract. The blood-brain relationship for stearic acid varies during development. Subcutaneously injected [1-^{14}C]-stearic acid is taken up by brain. Age-related changes in the metabolism of stearic acid have been determined in mouse brain from birth to maturity. Total lipid radioactivity reaches a maximum at 18 days of age and decreases afterwards until adulthood. However, specific radioactivity presents the highest value at 1 day of age and declines from then on. At any age, the injected acid is taken up and partly metabolized in the brain, either by elongation or by degradation *in situ* and resynthesis of new fatty acids; it is also desaturated, and the oleic acid thus formed is eventually elongated. The labeled stearic acid is incorporated into brain lipids with a different pattern according to the age of the injected animal.

Introduction

Saturated fatty acids are taken up by brain when either fed to [6] or injected into [3] animals. Subcutaneously injected stearic acid is taken up through the blood-brain barrier and is further incorporated into brain lipids [4, 12–14]. Stearic acid is the primer for very long chain fatty acids in brain [1, 2, 16]. This labeled acid is either directly incorporated into membrane lipids or first metabolized within the brain into longer chains (thus providing arachidic, behenic and lignoceric acids). It can also be degraded into acetate units and utilized for synthesis of medium chain fatty acids and cholesterol. Exogenous stearic acid is incorporated into subcellular particles when isolated as a whole [13]; after fractionation, it is found in myelin [12, 14, 17] and in synaptosomes [4]. Indeed, after direct intracerebral injection fatty acids are incorporated into brain lipids [19].

It has been known for several years that lipids such as cholesterol and its precursor acetate are readily taken up by the brains of young rats before myelination. The process begins around 12 days after birth, but the entry of these compounds is very much re-

duced in the brains of adult animals [1, 7, 11]. Thus, it seemed interesting to study the blood-brain relationship [8] during brain maturation for a saturated fatty acid. Previous studies have shown that the blood-brain barrier for inulin and chloride [20] to most compounds is more developed in the mature animal. The present study was undertaken to obtain the pattern of labeling of stearic acid in the developing brain from birth to adulthood, a period that involves important metabolic and structural changes associated with glial cell multiplication and myelination.

Materials and Methods

1 mCi [1-^{14}C]-stearic acid (51 mCi/mmol) was obtained from CEA (France) and neutralized with an equimolecular amount of NaOH (0.5 ml of a solution of 2 mg/ml of NaOH). Bovine albumin (0.5 ml, 0.5 μM) in 0.5% NaCl was added and the mixture was stirred vigorously.

C_{57}Bl/6J mice of both sexes were fed a standard diet of biscuits from Extra Labo (France). The amount of the injected stearic acid was determined by the body weight of the animal (10 μCi/g). The purity of the radio-labeled fatty acid was greater than 99.9%. As ascertained by gas-liquid radiochromatography, this material was free of homologous fatty acids. The animals were sacrificed 17 h after injection of stearate. At this time, for 15- and for 8-day-old mice, the slope of the distribution of activity is increasing as a function of time after subcutaneous injection of 1-^{14}C stearate. Brains were excised, washed, and sliced with a razor blade in isotonic solution (0.9% NaCl); the tissue fragments were spun down at 17,500 g for 10 min, and blood cells were discarded. The pellet was homogenized in a Potter-Elvehjem homogenizer using 30 ml isotonic solution/g of tissue, and centrifuged at 100,000 g for 60 min. The pellet contains all subcellular particles and the soluble fatty acids within the brain cells are discarded. Moreover, the contamination by blood is eliminated by this method. Total lipids were extracted by chloroform-methanol (2:1 v/v) [9, 10]. Radioactivity was counted in a Packard scintillation counter using PPO, POPOP and toluene. Lipids were isolated by thin-layer chromatography after elution with chloroform-methanol-water (70:30:4 v/v/v) [18]. Thin-layer chromatographs were prepared using Merck 60 F 254 silica gel; the various lipids were identified by co-chromatography with commercial standards. Under these conditions, sphingomyelin, inositol phosphatides (PI) + choline phosphatides (PC) + serine phosphatides (PS), sulfatides, cholesterol, and cerebrosides are well separated [1, 4]. The spots containing PI + PC + PS from the 70:30:4 migration were scraped and extracted 5 times with 3 ml/cm^2 chloroform-methanol 2:1. These lipids were further separated by phenol in water (436:100 v/v) and 10.5 M NH_4OH, (99:1 v/v) [17]. Because unesterified fatty acids are contaminated by ceramides, another solvent system was used to further purify them: chloroform-acetic acid (90:10 v/v). This system was employed with the total lipid extract. The spots were visualized by iodine, scraped, and counted in the presence of Cab-o-sil PPO and dimethyl POPOP. No quench correction procedure was applied since the same quantity of silica gel was scraped for each individual lipid, the quench factor thus remaining constant (iodine is evaporated before scraping).

Lipids from the total lipid extract are methylated [15]. Fatty acid methyl esters thus obtained were directly analyzed by gas-liquid chromatography. The gas-liquid chromatography is carried out on a SE 30 column at 175–265 °C, 2 °C/min, in a Hewlett-Packard 5750 with automatic counting of the eluate. During gas-liquid chromatography, 50% of the effluent gas is passed through a flame ionization detector in order to determine relative retention time of esters, while the remaining gas passes through a radioactivity monitoring system, with a 700 °C combustion oven, for the determination of radioactivity.

130 mice of various ages were used and each experiment was performed at least 3 times.

Results and Discussion

In figure 1, it can be seen that radioactivity in the total lipid extract (expressed as cpm per brain) increases up to 18 days of age and then decreases until adult age. Specific activity (expressed as cpm per mg of lipid extract) is maximum at 1 day of age and then decreases.

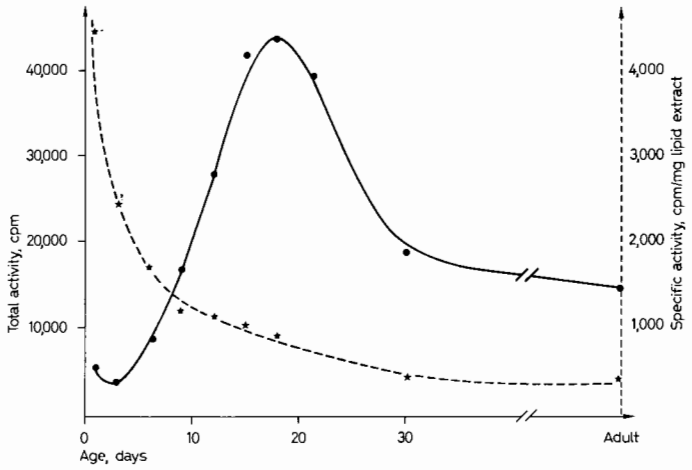

Fig. 1. Distribution of radioactivity in mouse brain lipids after subcutaneous injection of [1-^{14}C]-stearic acid as a function of age. ● = Total lipids after Folch wash (cpm), ★ = specific radioactivity (cpm/mg of lipid extract). Each point is the mean value of at least three experiments.

Table I. Distribution of relative radioactivity in membrane lipids after subcutaneous injection of [1-^{14}C]-stearate

Age of mice	Sphingo-myelin %	PC %	PI %	PS %	Sulphatides + phosphat-idic acid, %	PE %	Cere-brosides %	Free fatty acids %	Chol-esterol %	Front %
New born	2.2		43.2		2.3	20.8	0.6	11.8	13.1	3.7
5 days	1.5	26.8	3.1	13.5	1.3	21.4	0.7	10.9	15.0	3.3
6 days	1.5	29.3	1.4	13.1	1.6	22.9	0.9	10.7	14.1	2.8
9 days	1.9	31.8	2.8	7.7	2.7	22.5	1.4	10.1	14.5	2.4
12 days	2.2	33.0	2.5	7.6	2.8	23.0	2.2	9.5	13.8	2.4
15 days	2.5	33.4	4.1	8.3	2.9	23.1	4.5	9.3	10.5	2.6
18 days	2.9	30.1	3.5	10.9	2.0	23.6	3.3	8.1	10.3	2.5
21 days	2.5	31.2	1.5	14.8	1.5	23.8	3.0	8.5	11.1	2.4
30 days	2.6	30.5	1.8	14.4	1.4	22.0	1.7	11.1	9.9	2.3
Adult	2.7	30.3	2.5	11.6	1.4	22.1	1.5	15.1	8.5	2.3

Same remarks as in figure 1. The percentage refers to the radioactivity found in the total lipid extract.

It is interesting to note that the highest level of total activity coincides with the peak period of myelination, which, in mice, occurs at 18 days. However, the activity relative to the total content of brain lipids is maximum during the first day of postnatal life, possibly reflecting glial cell multiplication. Thus, during brain maturation, the uptake of stearic acid varies considerably.

Table I shows the relative percentage of radioactivity found in the major classes of lipids following a subcutaneous injection of [1-^{14}C]-stearate into mice of different ages. The radioactivity is present in all major components, indicating active incorporation into brain constituents. Most of the radioactivity in lipids is found in choline phosphoglycerides (PC), but the progression is not the same for

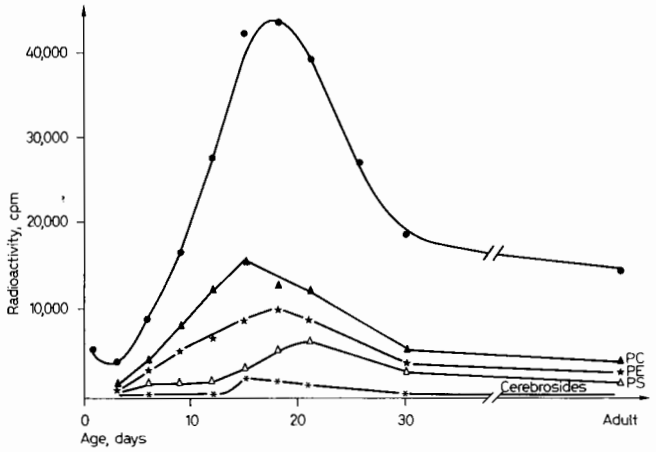

Fig. 2. Variations of radioactivity in the major classes of lipids as a function of age after subcutaneous injection of stearate. ● = Total lipids after Folch wash, ▲ = PC = choline phosphoglycerides, ★ = PE = ethanolamine phosphoglycerides, △ = PS = serine phosphoglycerides, ✳ = cerebrosides. The activity is expressed as the radioactivity (in cpm for either total lipid extract or each individual lipid).

each phospholipid. PC and inositol phosphoglycerides (PI) increase until 15 days of age and then decrease. Ethanolamine phosphoglycerides (PE) increase until 21 days of age and then decrease. Conversely, serine phosphoglycerides (PS) decrease until 15 days of age and then increase but again decrease in adults. The percentage of radioactivity found in cerebrosides, sulfatides, and sphingomyelin is maximum at 18 days of age. The percentage of free fatty acids is relatively constant at all ages. Some activity is found in cholesterol at all ages, suggesting that stearic acid is degraded to radioactive acetate, which is in turn used for the synthesis of cholesterol.

Figure 2 shows the progression of uptake of radioactivity into the major classes of lipids after injection of [1-^{14}C]-stearate. Phosphatidyl choline and cerebrosides show the maximum of activity at 15 days of age, PE at 18 days of age and PS at 21 days of age.

Table II shows the time course of the fatty acid profile in total lipids. We have previously demonstrated that systemically administered stearic acid is predominantly taken up directly from blood without prior degradation to acetate [13]. Therefore, all modifications observed reflect metabolism in the brain. At all ages studied, injected stearic acid was partly degraded to radioactive acetate; subsequent *de novo* biosynthesis or elongation of endogenous short chains within the brain explain the presence of C_{14} and C_{16} acids. The level of arachidic acid was low, but not negligible. This indicates that stearic acid is partly elongated. Oleate is also radioactive, thus confirming that the brain is able to produce oleic acid from stearate. Due to the elongation of oleate some radioactivity is found in monounsaturated very long chains. The brains of 18-day-old mice contain 1.4% of radioactive C_{24} after injection of stearate.

In conclusion, a long chain saturated fatty acid (stearic acid) is taken up by brain and incorporated into lipids, but the blood-brain relationship for stearic acid varies during brain development. Total lipid radioactivity (cpm) is maximum at 18 days of age but specific activity (cpm/mg of lipid extract) is maximum at 1 day of age. At all ages studied, stearic acid is partly metabolized in the brain either by elongation or by degradation and *in situ* resynthesis of fatty acids. It is also desaturated and oleate is eventually elongated.

Table II. Evolution of the metabolism of stearic acid as a function of age

Age of mice	C_{14}, %	C_{16}, %	C_{18}, %		C_{20}, %	
			saturated	unsaturated	saturated	unsaturated
3 days	1.2	9.8	77.1	8.2	2.3	0.7
6 days	1.5	15.0	75.9	7.5	trace	trace
9 days	1.1	13.4	76.3	6.5	1.3	1.3
12 days	2.0	16.7	66.4	11.6	1.7	0.6
15 days	1.0	20.8	67.5	5.9	3.1	1.6
18 days	trace	24.6	67.3	4.0	1.2	1.3
21 days	–	15.7	68.5	9.8	2.9	1.9
30 days	–	13.3	68.5	9.8	5.2	2.3

At least 10,000 cpm were injected at each gas-liquid chromatography. The percentage refers to the activity found in the total labeled fatty acids after methylation of the lipid extract. The brain of 18-day-old mice contains 1.4% radioactive C_{24}.

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