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# IN VIVO INCORPORATION OF EXOGENOUS [1-$^{14}$C]STEARIC ACID INTO NEURONS AND ASTROCYTES

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SUMMARY

Exogenous stearic acid is needed to synthesize the membranes of neurons and astrocytes. Subcutaneously injected [1-$^{14}$C]acid is taken up through the 'blood brain barrier' and incorporated into lipids of both cell types, the specific radioactivity being higher in astrocytes as compared to neurons (2200 and 800 cpm/mg proteins, respectively), 20 h after injection. Phospholipids contain high amount of radioactivity (80% in astrocytes, 65% in neurons); glycosphingolipids contain low quantities of label in the two cell types. The injected acid is partly metabolized in the brain by elongation and desaturation (thus providing very long chains, saturated mono-unsaturated and poly-unsaturated); it is also partly degraded into acetate units (utilized for synthesis of palmitic acid).

During its development, brain is actually dependent on exogenous fatty acids [2], as saturated fatty acids necessary for membrane formation are obtained endogenously or exogenously. Indeed saturated fatty acids are synthesized in the brain in various organelles [4]; however, radioactive long chains are taken up by brain either when fed [6] or injected [2]. The label of stearic acid, after subcutaneous injection, is incorporated into lipids of subcellular particles as such or after being metabolized in brain (by degradation and resynthesis in situ of fatty acids, or by elongation) [7]. So myelin [8] as well as synaptosomes [3] are using labeled stearic acid transported from blood to brain. This work was undertaken to study the abilities of neurons and astrocytes to take up this acid through the 'blood brain barrier'.

Subcutaneous injection of labeled albumin bound [1-$^{14}$C]stearic acid was performed as previously described (25 $\mu$Ci per animal) [7]. Mice

(15-day-old) were fed with standard diet biscuits from Extra Labo (France). Brains are excised, washed with buffer; neurons and astrocytes were obtained by trypsination, screening and density gradient [11]. The cells were washed several times in 0.32 M sucrose; protein content was determined according to Lowry [9] and the radioactivity was measured by liquid scintillation using Bray solution. Lipids were extracted by sonication (6 × 30 sec) and stirring for 30 min; they were further analysed by multiple two-dimensional thin layer chromatography [12]. After detection by iodine vapor, the spots were scraped and radioactivity was measured in the presence of Cab-o-sil. No quench correction was performed, as the same quantity of silicagel was scraped each time. Lipids from total lipid extract were methylated [10] and fatty acid methyl esters were analysed by gas-liquid chromatography on 3% SE 30 column, temperature programming 180°—280°C at 2°C/min; 1% of the effluent passed in the flame ionization detector, 99% was collected on anthracene and counted. Each experiment was performed at least three times. About 260 mice were injected for this study.

Using morphological criteria [13] neurons examined under phase contrast microscopy were distinguishable with their large nuclei and portions of thick processes. Astrocytes have typical thin highly branched processes. The purity of neuron and astrocyte fractions were about 90% and 70% respectively. Fig. 1 shows the uptake of radioactivity by neurons and astrocytes measured at different times after injection of [1-$^{14}$C]stearic acid and expressed in

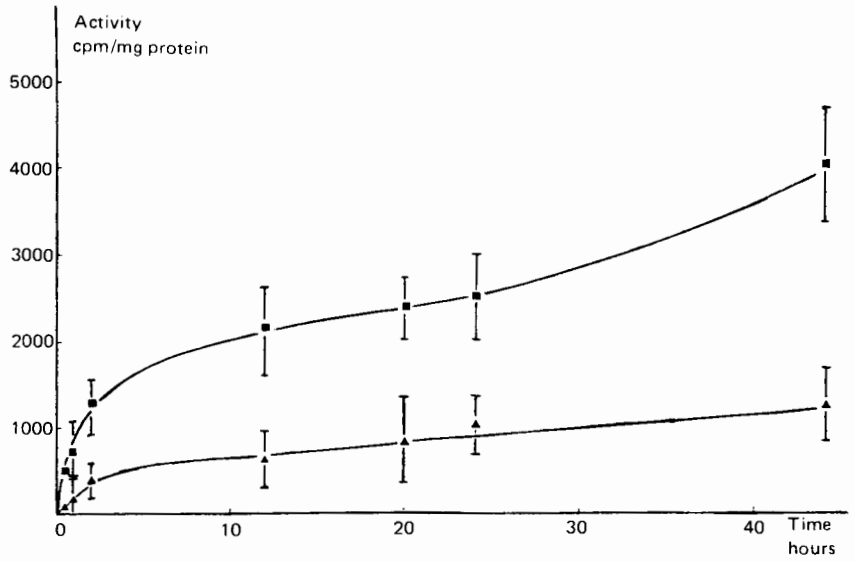


Fig. 1. Evolution of specific radioactivity (cpm/mg protein) in neurons (▲) and astrocytes (■), after subcutaneous injection of [1-$^{14}$C]stearate. Each point represents the mean value from 3 pools of 6 mice ± standard deviation.

cpm/mg proteins. The neuronal specific radioactivity increases up to 20 h and reaches a plateau from then on. In astrocytes, the values obtained are higher (as compared to neurons); they are rapidly increasing during the 2 h following injection and go on less prominently up to 40 h. Twenty hours after injection, the specific radioactivity is 2200 and 800 cpm/mg protein in astrocytes and neurons, respectively. Thus, astrocytes present higher metabolic activity as compared to neurons.

Table I shows the distribution of relative radioactivity in lipids from neurons and astrocytes. Twenty hours after injection most of the radioactivity in the lipids was found in phospholipids (80% in astrocytes and 65% in neurons). Comparing both cell types, it is interesting to note that phosphatidyl choline and sphingomyelin represent the same percentage; marked differences being found in phosphatidyl-ethanolamine and phosphatidyl-serine. It must be pointed out that both cell types have low amount of radioactivity in glycosphingolipids. Astrocytes contain lower quantities of labeled sulfatides and cerebrosides as compared to neurons.

Table II shows the profile of labeled fatty acids in astrocytic and neuronal lipids. Most of the radioactivity is found in stearic acid, indicating that albumin bound injected stearic acid is mainly incorporated in brain cells without modifications.

Results are consistent with the values of fatty acid uptake obtained in vitro with isolated neurons and astrocytes [5]. Previous studies [2] have shown that blood contains only [1-$^{14}$C]stearic acid. In total brain, chemical decarboxylation showed most of the radioactivity to be in the carboxyl end (65% 18 h after injection). Before being used for membrane elaboration, the

TABLE I

DISTRIBUTION OF RELATIVE RADIOACTIVITY IN LIPIDS FROM NEURONS AND ASTROCYTES, 20 h AFTER SUBCUTANEOUS INJECTION OF [1-$^{14}$C]STEARATE

pc, phosphatidyl-choline; pe, phosphatidylethanolamine; ps, phosphatidyl-serine; pi, phosphatidyl-inositol; sph, sphingomyelin; pa, phosphatidic acid; cer, cerebrosides; sulf, sulfatides; front, cholesterol, cholesterol esters and free fatty acids. Mean values from 3 experiments (pools of 20 mice) ± standard deviation.

| | Neurons (%) | Astrocytes (%) |
|---|---|---|
| pc | 39.1 ± 3.0 | 37.9 ± 3.4 |
| pe | 17.1 ± 3.6 | 22.7 ± 2.5 |
| ps | 4.4 ± 2.6 | 9.5 ± 1.4 |
| pi | 7.3 ± 2.2 | 9.9 ± 2.0 |
| sph | 6.6 ± 1.4 | 7.6 ± 2.6 |
| pa | 1.8 ± 1.8 | 1.1 ± 0.6 |
| cer | 3.7 ± 3.8 | 1.3 ± 1.3 |
| sulf | 3.3 ± 3.6 | 0.8 ± 0.2 |
| front | 17.6 ± 3.1 | 9.0 ± 2.4 |

TABLE II

DISTRIBUTION OF RELATIVE ACTIVITY IN FATTY ACIDS FROM NEURONS AND ASTROCYTES LIPIDS, 20 h AFTER SUBCUTANEOUS INJECTION OF [1-$^{14}$C]STEARATE.

tr, traces < 1%. Mean values from 3 experiments (pools of 20 mice) ± standard deviation.

| | Neurons (%) | Astrocytes (%) |
|---|---|---|
| C 16:0 | 29.0 ± 1.0 | 23.2 ± 2.8 |
| C 16:1 | tr | tr |
| C 17:0 | 2.5 ± 0.4 | tr |
| C 18:0 | 45.3 ± 2.3 | 46.5 ± 7.2 |
| C 18:1 | 13.8 ± 1.3 | 15.4 ± 2.0 |
| C 20:0 | 1.6 ± 0.8 | tr |
| C 20:1 | 1.8 ± 2.5 | 2.0 ± 1.4 |
| C 20:4 ω 6 / 20:3 ω 6 | 3.2 ± 0.1 | 4.2 ± 1.8 |
| C 22:0 / 22:1 | tr | 1.9 ± 1.1 |
| C 22:5 ω 6 / 22:6 ω 3 | tr | 2.1 ± 1.4 |
| C 24:0 / 24:1 | tr | 1.6 ± 2.3 |

injection acid is partly desaturated in brain with an equal rate in both cell types; very long polyunsaturated chains are also produced in higher quantities in astrocytes as compared to neurons. The occurrence of low amounts of lignoceric and nervonic acid in astrocytes is of interest. The injected acid is also degraded inside brain in acetate units, utilized for synthesis of medium chain fatty acids as palmitic acid. Thus exogenous stearic acid is needed to synthesize the membranes of astrocytes and neurons, this effect is probably more important during cell multiplication as brain takes up very actively this fatty acid during this period [1].

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