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IN VIVO INCORPORATION OF EXOGENOUS STEARIC ACID IN SYNAPTOSOMES: HIGH OCCURRENCE OF NON-ESTERIFIED FATTY ACIDS

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SUMMARY

Subcutaneously injected stearic acid is taken up by the brain through the 'blood-brain barrier' and is incorporated into synaptosomal lipids (as well as into other brain compartments). Phospholipids are potent acceptors (choline phosphatides, ethanolamine phosphatides, serine phosphatides). Moreover a high level of radioactivity was found in non-esterified fatty acids.

The suggestion has been made that neurotransmitter-modulated release of non-esterified fatty acids may be part of the mechanism for controlling membrane permeability [13]; non-esterified fatty acids have been found in the cerebral cortex associated with proteolipids, including the cholinergic receptor [14]. β-Bungarotoxin, a presynaptic neurotoxin, is a potent phospholipase A2, thus showing that its action proceeds through the release of fatty acids [17].

Saturated fatty acids are taken up by brain either when fed [6] or injected [3]. Subcutaneously injected stearic acid is taken up through the blood-brain barrier and is further incorporated into brain lipids [10,11]. This labelled acid is either directly incorporated into membrane lipids or metabolized inside the brain into longer chains (thus providing arachidic, behenic and lignoceric acids). It can also be degraded in acetate units, utilized for the synthesis of medium-chain fatty acids and cholesterol. However, a small amount remains as free fatty acid. Exogenous stearic acid is incorporated into subcellular particles when isolated as a whole, and also in myelin after fractionation [10]. This study was undertaken to examine the possible incorporation of exogenous fatty acids into brain synaptosomes.

[1-^{14}C]Stearic acid (50 μCi) (neutralized with equimolar amount of NaOH and solubilized by lipid-free bovine albumin) was injected to ten 18-day-old mice as previously described [10]. Animals were fed with standard diet bis-

cuits from Extra Labo (France). Mice were sacrificed 1 h after injection. Synaptosomes were isolated by a modification of the techniques of Whittaker et al. [18] and Israel and Frachon-Mastour [12]. The forebrains were homogenized in 10% (w/v) sucrose in a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 1500 × *g* (Sorvall RC2B). The supernatant was spun down at 10,800 × *g* for 30 min; the pellet thus obtained was suspended in 0.32 M sucrose and layered on discontinuous sucrose gradients made of 1.2 M sucrose (13 ml) and 1.0 M sucrose (10 ml) [5]. These gradients were then centrifuged for 2 h at 80,000 × *g* (21,000 rpm in a Spinco L565 with a rotor Sw27). The synaptosomal layers which formed at the interface of the 1.2—1.0 M sucrose solutions were then collected and combined synaptosomal layers were diluted very gently with cold distilled water and spun down at 25,300 × *g* for 30 min. Their purity was checked by electron microscopy.

Total lipids were extracted with chloroform-methanol 2:1 (v/v). After Folch partition [8,9], lipids from the lower phase were isolated on thin layer chromatograms after migration with chloroform-methanol-water (70:30:4, v/v/v) [16]. The spot containing the main phosphatides (inositol, choline and serine) was scraped, extracted and further separated into each constituent by phenol in water (436:100 w/v)—10.5 M NH_4OH, 99:1 v/v [15]. As non-esterified fatty acids are mainly contaminated by ceramides another system was used: chloroform-acetic acid (90:10 v/v). In the same way, lipids from the upper phase were analyzed by thin layer chromatograms, the migrating solvent being chloroform-methanol-0.25% $CaCl_2$ (65:35:8 v/v/v) [7]. The spots were visualized by iodine, scraped and counted in the presence of Cab-o-sil, PPO and dimethyl-POPOP. Lipids were eventually methylated and fatty acid methyl esters were analyzed by gas liquid chromatography with automatic counting of the eluate [2]. Each experiment with 10 mice was performed at least four times.

In Table I are summarized the main parameters obtained. About 40% of the lyophilized synaptosomes is made of lipids. The relatively high radioactivity in the upper Folch phase must be noted as this fraction contains gangliosides (and proteolipids). Table II shows the percentage of the radioactivity in various lower phase lipids. Of the radioactivity 60% is present in all major lipids, indicating active incorporation into synaptosomal lipids; among the phospholipids, choline phosphatides present the highest radioactivity, followed by ethanolamine phosphatides and serine phosphatides. Of the total radioactivity 0.8% was found in cholesterol esters, cerebrosides, ceramides, monogalactosyl diglycerides and some unidentified spots. The very high radioactivity found in unesterified fatty acids must be pointed out: about 30% of the total radioactivity in the lower phase is found in these molecules. (Moreover, 29.8% of the radioactivity of the upper Folch wash is found in non-esterified fatty acids, 48.5% being in gangliosides and the remaining staying at the origin, possibly covalently bound to proteolipids.)

Fatty acid analysis shows that most of the radioactivity is found in stearic acid (75%), some activity also being found in palmitic acid, oleic acid and

TABLE I

RADIOACTIVITY INCORPORATED INTO SYNAPTOSOMES PER BRAIN

Values are means ± S.D. of four experiments.

Synaptosomal total lipid extract (mg)	6.1 ± 1.1
Total radioactivity in total lipid extract (cpm)	2947 ± 559
upper Folch wash (cpm)	425 ± 121
lower Folch wash (cpm)	2561 ± 546

polyunsaturated fatty acids with 20 and 22 carbon atoms. It is not known if these acids are derived from exogenous stearic acid by metabolism inside synaptosomes or if they are metabolized in another compartment (neuronal pericaryon for instance) and fixed thereafter in nerve endings.

When compared to other membranes, the content of non-esterified fatty acids is much higher in synaptosomes as expressed per total mg lipids. Relative to synaptosomes, 26% is found in myelin, and 28% in total membranes.

It remains unknown whether fatty acids in synaptosomes derive from axonal flow or originate locally by non-specific adsorption or active uptake [1].

The high content of the total radioactivity as non-esterified fatty acids (about 36% of the total radioactivity) is possibly correlated with synaptic transmission as these acids have been found in cerebral cortex associated with proteolipids, including cholinergic receptors [13], and the neurotransmitter-modulated release of unesterified fatty acids may be part of the mechanism for controlling membrane permeability [14].

This study shows that synaptosomal fatty acids are partly coming from

TABLE II

DISTRIBUTION OF RADIOACTIVITY AFTER SUBCUTANEOUS INJECTION OF [1-^{14}C]STEARIC ACID

Values are means ± S.D. of four experiments.

Lipids	%
Non-esterified fatty acids	29.6 ± 3.2
Choline phosphatides	27.0 ± 5.2
Ethanolamine phosphatides	17.7 ± 0.5
Serine phosphatides	13.0 ± 3.1
Cholesterol	6.0 ± 0.8
Inositol phosphatides	2.4 ± 0.1
Sphingomyelin	1.7 ± 0.5
Phosphatidic acid + sulphatides	1.7 ± 0.4
Total	99.1

the blood through the blood-brain barrier in addition to synthesis in situ. Although the brain is able to synthesize its own fatty acids [4], the relative amount of fatty acids in synaptosomes due to endogenous synthesis is not known, so far.

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