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n-3 PUFA status affects expression of genes involved in neuroenergetics differently in the fronto-parietal cortex compared to the CA1 area of the hippocampus: Effect of rest and neuronal activation in the rat ☆

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ABSTRACT

n-3 Polyunsaturated fatty acids (PUFA) support whole brain energy metabolism but their impact on neuroenergetics in specific brain areas and during neuronal activation is still poorly understood. We tested the effect of feeding rats as control, n-3 PUFA-deficient diet, or docosahexaenoic acid (DHA)-supplemented diet on the expression of key genes in fronto-parietal cortex and hippocampal neuroenergetics before and after neuronal stimulation (activated) by an enriched environment. Compared to control rats, n-3 deficiency specifically repressed GLUT1 gene expression in the fronto-parietal cortex in basal state and also during neuronal activation which specifically stimulated GLUT1. In contrast, in the CA1 area, n-3 deficiency improved the glutamatergic synapse function in both neuronal states (glutamate transporters, Na^+/K^+ ATPase). DHA supplementation induced overexpression of genes encoding enzymes of the oxidative phosphorylation system and the F1F0 ATP synthase in the CA1 area. We conclude that n-3 deficiency repressed GLUT1 gene expression in the cerebral cortex, while DHA supplementation improved the mitochondrial ATP generation in the CA1 area of the hippocampus.

1. Introduction

Brain function requires about 20% of resting metabolism in humans, mostly for action potentials and postsynaptic currents [1]. Blood glucose is the major energy substrate and its consumption by the brain is closely related to local neuronal activity. The two isoforms (endothelial and astrocytic) of glucose transporter, GLUT1, are key regulators of brain glucose consumption and of neuroenergetics. Their local density consistently parallels local rates of glucose utilization [2]. Increased hippocampal energy demand during activation of memory processing has been shown to be met through increased GLUT1 gene and protein expression [3]. Moreover, a process of neurometabolic coupling termed astrocyte-neuron lactate shuttle (ANLS) has been demonstrated at the level of the glutamatergic synapse. This pathway is triggered by the activation of glycolysis in astrocytes, resulting in the production and release of lactate that may be utilized by neurons in addition to glucose [4,5]. In the adult brain, the main events of glutamatergic neuroenergetics can be summarized as shown in Fig. 1.

One of the main metabolic functions of dietary n-3 polyunsaturated fatty acids (PUFA) is to support docosahexaenoic acid (DHA, 22:6n-3) accretion in phospholipid membranes and to ensure proper brain development [6,7]. Data from animal studies have shown that perinatal n-3 chronic deficiency decreases DHA in brain membranes and impairs monoaminergic and glutamatergic synaptic function, leading to deficits in learning and memory behavior [8–10]. Such alterations in synaptic functions, which use most of the brain energy, could result from brain glucose hypometabolism. We previously showed that glucose utilization, i.e. GLUT1 immunostaining and protein amounts of glucose transporters GLUT1, is lower in the cerebral cortex of rats deprived of n-3 PUFA [11–13]. One positron emission tomography study suggests a positive correlation between plasma DHA and

Abbreviations: ANLS, astrocyte-neuron lactate shuttle; AA, arachidonic acid; DHA, docosahexaenoic acid; n-6DPA, n-6docosapentaenoic acid; LA, linoleic acid; LC-PUFA, long chain PUFA; LNA, α-linolenic acid; PUFA, polyunsaturated fatty acid.

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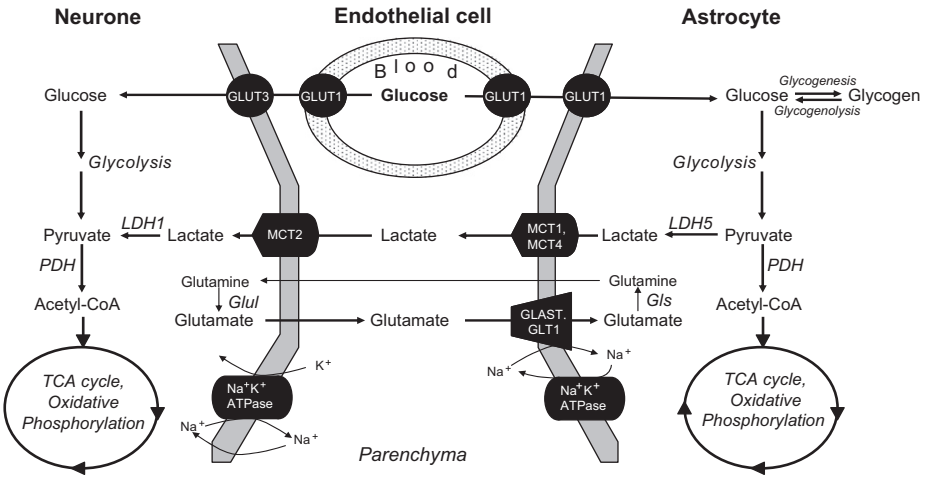

Fig. 1. Glutamatergic neuroenergetics depicted in the astrocyte-neuron lactate shuttle hypothesis (ANLSH) in the adult brain (adapted from [5]). In the basal condition, glucose is translocated from blood via the brain endothelial glucose transporter GLUT1 (55 kDa isoform), and is taken up by astrocytes via GLUT1 (45 kDa isoform encoded by the same gene encoding for endothelial GLUT1) and by neurons via GLUT3. Glucose is metabolized via glycolysis and tricarbocylic acid cycle (TCA) to produce ATP in conjunction with oxidative phosphorylation. In astrocytes, part of the pyruvate produced from glucose is converted to lactate via the lactate dehydrogenase 5 (LDH5) isoform. Lactate is then released in parenchyma involving the specific monocarboxylate transporters 1 and 4 (MCT1 and 4) isoforms of astrocytes and taken up by neurons via MCT2 isoform and converted to pyruvate (LDH1 isoform) for further metabolization by TCA cycle. Upon glutamatergic activation, the increase of ATP consumption due to the increase of neuronal Na^+/K^+ ATPase activity needs to be compensated for by an increased utilization of glucose as energy substrate. Moreover, glutamate, after its release in the synaptic cleft, is transported into perisynaptic astrocytes via specific transporters (GLAST and GLT1) in which activity is coupled to Na^+ influx. The entry of Na^+ activates the astrocytic Na^+/K^+ ATPase, favoring the utilization of glucose and the production of lactate that is released to neurons. Glycogenolysis could sustain glucose utilization by astrocytes during prolonged activation. Finally, glutamate may be shuttled back from perisynaptic astrocytes to neurons via the glutamate–glutamine cycle (glutamine synthesis by astrocytic glutamine synthase (Gls), and glutamate synthesis by neuron by glutaminase (Glul)). Glutamine may also serve as a neuronal energy substrate.

glucose metabolic rates in the frontal and temporoparietal cortices of depressed subjects [14]. Other key steps in neuroenergetics may be altered by n-3 PUFA status such as those of the mitochondrial oxidative phosphorylation pathway. Indeed, a reduction of cytochrome oxidase activity in frontoparietal cortex and hippocampus was reported in n-3 deficient rats [11], whereas rats fed high n-3 PUFA diets exhibited an upregulation of the expression of genes encoding for subunits of cytochrome c oxidase, cytochrome b and ATP synthases in the total brain [15].

Dietary n-3 PUFA can also influence brain neuronal activity by modulating physical properties and functions of membranes, and cell signaling and gene expression via nuclear transcription factors [16,17].

In the present study, we hypothesized that DHA may also affect brain energy metabolism by modulating the gene expression of key proteins in neuroenergetics, notably that of GLUT1. To address this question, rats were raised from weaning on a diet deprived or supplemented in n-3 PUFA [13] and were tested in a basal state or during neuronal activation after exposition to an enriched environment conceived for stimulating their exploratory activity. The cerebral activation mainly recruits glutamatergic synapses in two brain areas involved in normal cognitive function, the frontal cortex and the CA1 area of the hippocampus. A multiple gene expression analysis was performed on main key metabolic genes (described in Fig. 1) in these two brain areas by using the reproducible and robust real-time PCR TaqMan Low-Density Array (TLDA) technique [18]. The fatty acid content in the main phospholipid classes, phosphatidylcholine (PC), ethanolamine glycerophospholipids (EPG) and phosphatidylserine (PS), was analyzed in the two brain areas.

2. Material and methods

2.1. Animals and diets

All animal experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Two weeks before mating, 30 adult pre-experimental female rats from the Wistar strain received 6.6% lipid diets (w/w) supplying either an adequate amount of n-3 PUFA as α-linolenate (0.67 energy% LNA, control group), a low amount of n-3 PUFA (0.01 en% LNA and 0 en% DHA, n-3 Deficient group) or high amount of n-3 long-chain (LC) PUFA, consisting in DHA for the major part (1.11 en% DHA, n-3 Supplemented group; Table 1). The fats of the different diets were made of oils blended as follows: control diet, high oleic acid–high linoleic acid sunflower oils (Lesieur, Asnière sur Seine) mixed (w/w) with rapeseed oil (Lesieur); n-3 deficient diet, high oleic acid–high linoleic acid sunflower oil as the sole source of fats; n-3 supplemented diet, 66% high oleic acid–high linoleic acid sunflower oils mixed with 34% tuna oil (Polaris). To minimize oxidative damage to PUFA, diets were prepared for two months at a time, stored at 4 °C in sealed bags and administered daily. At weaning (3 week of age), 44 males were housed two per cage and fed for 6 week the same diet as that of their mothers ($n=20$/control group, and $n=12$/other diet groups). Animals were placed on a 12 h light–dark cycle, with free access to food and water. At 9 week of age, rats from each group of diet were randomly designed to basal state or to neuronal stimulation: the first group was left at rest in standard cages (basal rats, $n=6$/diet group) while the second group was placed (as described below) in an enriched environment (activated rats, $n=6$/diet group). At the end of the exposition procedure, rats were killed by decapitation and the fronto-parietal cortex and the CA1 area of the hippocampus were rapidly dissected on ice, and separated into two pools: one was frozen in liquid nitrogen, and stored at −80 °C until used for analysis of fatty acids; the other was stored at −20 °C in RNA later (RNA Stabilization Reagent, Qiagen) until quantification of mRNA.

2.2. Enriched environment and c-Fos induction

Exposition to the enriched environment was carried out for each rat in a single session during 1 h in a customized box (0.8 m × 0.9 m × 0.6 m, sheltering 3 rats per box) with several objects arranged on the floor which stimulated their exploratory behavior (toys, ball, plastic tubes, ladders, sleeping quarter) [19]. The effectiveness of neuronal activation in the frontal cortex and the CA1 region was