INTRODUCTION AND AIMS OF THE STUDY

Second to the adipose tissue, brain is the most lipid concentrated organ in the body. More concretely, brain is very rich in two long chain polyunsaturated fatty acids (LcPUFA), arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), derivatives of linoleic acid (LA, 18:2n-6) and α-linolenic acid (LNA, 18:3n-3), respectively. All of them play vital roles in the organism but, only LA and LNA are considered as essential since they cannot be synthesized, being the diet the only source. However, the synthesis rate of AA and DHA from their parent fatty acids is too slow to cover body requirements, especially in stages with high lipid metabolism like development, and is for this reason why the two fatty acids must be present in the diet as well. Hence, an appropriated dietary lipid composition is important first, for a proper development and later on, for the maintenance of the organism functions.

A large number of PUFA-deficient dietary studies were carried out the last decades, all of them pointed out to the necessity of PUFA for a proper development of the central nervous system. In addition, while PUFA-deficient diets have been implicated with impairment or completely lost of cognitive functions, PUFA-enriched diets have been related with an improvement of the learning capacity. Thus, it is clear that PUFA are somehow involved in memory and learning processes although the exact mechanism how they act is still poorly understood.

The hypothesis more studied is the influence that dietary lipids, as future components of membrane phospholipids, might have on the membrane fluidity. This biophysical property is determined by different factors, like temperature, cholesterol level and, phospholipid composition. Since the body temperature in mammals is constant, alterations in membrane lipid composition might be considered as the responsible of changes in the fluidity and, consequently in whole cell function. The first hints of the active role played by the lipids in the cell functioning, were provided by experiments showing that the activity of a number of transmembrane and membrane-

associated enzymes was modified by the membrane fluidity. Importantly, among these enzymes, are those implicated in signal transduction.

However, processes like behavioral changes, learning, loss of memory and, aging are too complex to be explained by a simple fluidity-dependent mechanism. Thus, more elements are needed for a better comprehension of the role that PUFAs are playing in such processes. Two relatively recent discoveries might help to shed some light on this topic: the lipid rafts and the fatty acid interaction with the peroxisome proliferator activated receptors (PPAR).

Lipid rafts are membrane subdomains with a specific lipid and protein composition. PUFA have been shown to change lipid raft composition and therefore, the activity of the proteins embedded in them may also be altered. On the other hand, PPAR are ligand-dependent nuclear factors that act as transcription factors binding directly to some specific sequences in the DNA chain. Interestingly, fatty acids and some lipid derivates were found to be ligands of these receptors. Therefore it is likely to link diet with gene expression patterns. All this information open a wide range of possible mechanisms through which the effects and the need for PUFAs could be better understood.

The present thesis attempts to provide new data that highlight the importance of dietary polyunsaturated fatty acids in mammalian brain metabolism and activity, and that might serve to establish a new mechanism to explain the fundamental role that PUFA display in the brain.

In this view the objectives were as follows:

1. To investigate the capacity of neuronal membranes to accumulate PUFAs and the relation of this accumulation with the dietary lipid composition.
2. To determine the importance of starting time and duration of feeding period.

3. To examine the changes in the membrane at the molecular level using gas chromatography, to analyze changes in total fatty acids and HPLC for changes in molecular species composition.
4. To reveal the relationship between age and phospholipid turnover in the neuronal membranes.
5. To investigate whether it is possible to restore PUFAs levels lost during aging process and to correlate changes in membrane composition with improvement of learning abilities.
6. To compare the phospholipid composition in forebrain, cerebellum and hippocampus and their response to diet.
7. To confirm the involvement of dietary lipids in brain gene expression and to reveal the importance of the exact lipid composition of the diet.
8. With the synthesis of the results obtained from the experiments, to find generalities regarding the role of polyunsaturated fatty acids in brain function and metabolism.

RESULTS

A. Changes in brain phospholipid fatty acid and molecular species composition.

This study was focused on the changes produced in the fatty acid and molecular species composition of the phosphatidylethanolamine. This phosphoglyceride is the most sensitive to change in the dietary lipids. In addition the propensity of this molecule to adopt inverted phases in the membrane, give to this particular phosphoglyceride an important role in the maintenance of the membrane fluidity.

In most of the studies on dietary lipids, animals fed with essential fatty acids (EFAs) deficient diets are involved. The capacity of the brain to conserve the DHA level is well known and usually two generations is needed to decrease its level

significantly. Situation seems to be different when PUFA-enriched diets are given to the animals due to the significant changes in DHA and AA levels observed in the forebrain when rats were fed since conception. This would suggest that brain prefers to accumulate PUFAs rather than to deplete them.

The diets exerting major changes on membrane phospholipid composition were the DHA–enriched diet and the LA/LNA in a ratio of 4.7:1 diet. Diets enriched in LA or with a ratio LA/LNA=2.9:1 did not have significant effects on the membrane phospholipid composition.

Regarding the molecular species composition, there was an increase in DHA-containing species while the AA-containing species decreased. This a consequence of the competence existing between the two families of fatty acids, n-3 and n-6, for the elongation and desaturation enzymatic machinery. It is remarkable that the decrease in AA-containing species was less pronounced when the diet given was LA/LNA=4.7:1.

The levels of AA and DHA were lower in the aged rats than in the younger ones. However, it was shown that these levels are susceptible to change when the diet is enriched in PUFAs. After only one month of feeding, the levels of DHA in both groups, the 3-month and the 2-year-old rats, were restored to similar levels present in the young rats.

The membrane lipid composition of the brain regions studied responded similarly to the different diets. The most striking result was that each region presents a

specific molecular species distribution that is maintained within a narrow range, despite the different experimental condition.

B. Effects of DHA-enriched diet on the cognitive functions.

One month of DHA-enriched diet was enough to restore the DHA membrane levels in brains of aged rats to the levels observed in the young rats. Despite this fact only the 3-month-old rats showed an improvement in the learning capacities. Two-year-old rats after one month feeding, did not perform better indicating that longer time of feeding are needed to alter the learning abilities.

C. Gene expression analyses.

Previous work done in this laboratory showed that DHA and LNA enriched diets altered the gene expression in the brain of rats fed since conception: 55 genes were overexpressed and 47 were repressed. Analysis of the gene expression in rats fed with the diet LA/LNA=4.7:1 showed that 20 of those 55 genes presented a higher overexpression. This would indicate that the gene expression patters are sensitive to the dietary lipid composition.

SUMMARY AND CONCLUSIONS

A. From the brain phospholipid analyzes it can be concluded that:

1. The significant changes observed in the levels of DHA and AA-containing molecular species in rats fed from conception indicates that the brain has a faster response to diet in PUFA-sufficient conditions.

2. The presence of DHA or an adequate ratio of n-6 to n-3 fatty acids in the diet is necessary to obtain a significant accumulation of DHA-containing species.

3. The accumulation of DHA is possible in aged animals, indicating that losses in PUFAs characteristic of the aging process can be reversed by an adequate diet.

4. Forebrain, cerebellum and hippocampus have a characteristic lipid composition and the three regions responded similarly to the diets.

5. It's the ratio of the molecular species, and not their absolute level, that the brain tries to maintain.

B. Although aged animals accumulated DHA up to the levels of the younger rats, they did not perform better in the learning test. Based on this observation it can be proposed that:

1. DHA does not only regulate the biophysical properties of the membrane, but it also participates in another mechanism, that would account for the improvement in learning. This mechanism would need a longer time to be affected by the changes in DHA levels. One of the possible mechanisms used by the DHA could be the regulation of the gene expression.

C. The analysis of brain gene expression in animals fed with LA/LNA 4.7:1 diet showed differences compared to the previous experiments, when rats where fed with fish and perilla oil (114). 20 genes showed a higher overexpression and some of them are involved in lipid metabolism, energy household and neurotransmission. Therefore it can be concluded that:

1. Regulation of the gene expression in the brain is sensitive to alteration in the dietary lipid composition.

On the basis of the presented results we propose that alteration in membrane architecture and function, due to the accumulation of DHA-containing molecular species, coupled with alterations in gene expression profiles may contribute to the observed beneficial impact of n-3 type polyunsaturated fatty acids on cognitive functions.

LIST OF PUBLICATIONS, POSTERS AND ORAL PRESENTATIONS

List of publications

1. Gwendolyn Barceló-Coblijn, Klára Kitajka, László G. Puskás, Endre Hőgyes Agnes Zvara, László Hackler jr., Tibor Farkas "Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio" *Biochem. Biophys. Acta* **1632** (1-3), 72-79, 2003.

2. László G. Puskás, Klára Kitajka, Csaba Nyakas, Gwendolyn Barceló-Coblijn, and Tibor Farkas. "Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus" *Proc. Natl. Acad. Sci* USA **100**, 1580-1585, 2003

3. Klára Kitajka, László G. Puskás, Agnes Zvara, László Hackler, Jr., Gwendolyn Barceló-Coblijn, Youn K. Yeo, and Tibor Farkás. "The role of n-3 polyunsaturated fatty acids in brain: Modulation of rat brain gene expression by dietary n-3 fatty acids" *Proc. Natl. Acad. Sci* USA **99**, 2619-2624, 2002.

Posters:

- "Reversal by fish oil of age induced alterations in molecular composition of rat brain phospholipids", Gwendolyn Barceló-Coblijn, Endre Hőgyes, Klára Kitajka, László G. Puskás, Csaba Nyakas and, Tibor Farkas, presented at the *"43rd International Conference on the Bioscience of Lipids"* held in Graz, Austria, (2002).
- "Molecular composition of phospholipids and gene expression in rat brain are affected by dietary fatty acids" Gwendolyn Barceló-Coblijn, László G. Puskás,

Klára Kitajka and, Tibor Farkas, presented at the *"8th International Congress on Phospholipids"*, held in Vienna, Austria (2002).

- "Factors controlling molecular composition and biophysical properties of vertebrate brain" Gwendolyn Barceló-Coblijn, Elfrieda Fodor, Klára Kitajka and, Tibor Farkas, presented at the *"27th FEBS/PABMB meeting"* held in Lisboa, Portugal (2001).

Oral presentation:

- "Gene expression and Molecular Composition of Phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio", given at the *"5th International Congress on Essential Fatty Acid and Eicosanoinds"*, Taipei, Taiwan (2002).