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Lysophosphatidylcholine as a Preferred Carrier Form of Docosahexaenoic Acid to the Brain

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Abstract

The metabolic fate of docosahexaenoic acid (DHA) was evaluated from its intake as a nutrient in triglycerides and phosphatidylcholines to its uptake by target tissues, especially the brain. Several approaches were used including the kinetics and tissue distribution of ingested ^{13}C-labeled DHA, the incorporation of radiolabeled DHA injected as its nonesterified form compared to the fatty acid esterified in lysophosphatidylcholine (lysoPC), and the capacity of the two latter forms to cross a reconstituted blood-brain barrier (BBB) consisting of cocultures of brain-capillary endothelial cells and astrocytes. The results obtained allow us to raise the hypothesis that lysoPC may represent a preferred physiological carrier of DHA to the brain.

Index Entries: Blood-brain barrier; fatty acid transport; lysophospholipids; Omega-3 fatty acids.

Introduction

Docosahexaenoic acid (DHA) is considered as the end-metabolite of the n-3 fatty acid family (Sprecher et al., 1999), although longer homologs have been described in the bovine retina (Aveldano and Sprecher, 1987). The retina is especially rich in DHA with some glycerophospholipids containing two DHA residues (Bisogno et al., 1999). In addition to the brain, where DHA is known as a major polyunsaturated fatty acid (PUFA) together with arachidonic acid (AA), DHA is predominant in spermatozoa and may specifically accumulate in the heart when present in the diet (Salem, 1988). It is then noteworthy that DHA exhibits some propensity for excitable membranes.

The sources of DHA are dual. The fatty acid may be directly provided by food, especially of marine origin, and synthesized from its food precursors (Horrocks and Yeo, 1999). It is generally assumed that DHA is less easily mobilized from tissues when compared with AA, presumably because cytosolic phospholipase A_2 involved in stimuli-activated cells is rather specific for arachidonoyl-containing glycerophospholipids (Dennis, 1997). Indeed, the brain is well-known to retain DHA as far as possible, even in n-3 fatty acid deficiency (Gazzah et al., 1995). This reinforces the concept that DHA is functionally important in some tissues like the brain. However, the molecular basis of its requirement in those tissues is largely unknown. Also, the mechanisms by which DHA is taken up to accumulate in those tissues, in particular the brain, are still unclear. The present article provides evidence for a preferential involvement of lysophosphatidylcholine (lysoPC) in DHA compartmental metabolism.

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Chemical Forms of DHA in Serum Albumin

Previous studies showed us that unsaturated fatty acids, especially AA and DHA, were better taken up by the developing rat brain when injected esterified in lysoPC compared to the nonesterified form, both forms being bound to albumin (Thies et al., 1992, 1994). We used then different approaches to investigate whether lysoPC could be a relevant chemical form available for DHA accretion in the brain.

One of the questions we asked was the biological relevance of DHA esterified in lysoPC. Interestingly, out of the 2.5 μ*M* DHA in human or rat plasma, 55% is esterified in lysoPC, the majority being associated with albumin, the remaining part (45%) is in the nonesterified form also bound to albumin. Dealing with AA, the predominance of lysoPC is even higher and consists of 80% of the 7 μ*M* in plasma (Croset et al., 2000). An important issue for the metabolic fate of such lysoPC in tissues is the ester position as PUFA are supposed to be mainly located at the sn-2 position of glycerophospholipids. However, the evaluation of 1-lyso,2-acyl-glycerophosphocholine (1-lyso,2-acyl-GPC) is not easy because of the propensity of the 2-acyl moiety to migrate to the sn-1 position during the sample treatment (Croset et al., 2000; Polette et al., 1999). The use of appropriate internal standards and an acidic pH to minimize the sn-2/sn-1 isomerization allowed us to evaluate that 1-lyso,2-acyl-GPC and 1-acyl,2-lyso-GPC were present at similar concentrations in plasma (Croset et al., 2000). It may be then hypothesized that 1-lyso,2-acyl-GPC, especially 1-lyso,2-AA/DHA-GPC, circulate and may be available for uptake by tissues including the brain.

Bioavailability of DHA from Triglycerides and Phosphatidylcholines

Data are available on the accumulation of DHA in different tissues especially in plasma-lipid pools after dietary supplementation (Harris, 1989; Nordoy et al., 1991; Hodge et al., 1993), but very few if any data on the dynamics of DHA distribution within those pools have been obtained. We have conducted some investigations in human and rat after intake of a single dose of DHA labeled with ^{13}C in which we have measured the ^{13}C enrichment as a function of time in different plasma-lipid pools and in the brain of rats.

In a first set of experiments, ^{13}C-labeled triglycerides containing around one-third of DHA were ingested by human volunteers and the ^{13}C-enrichment in DHA from various lipid compartments of blood plasma, platelets, leukocytes, and erythrocytes was evaluated by gas chromatography combustion-isotope ratio mass spectrometry (GCC-IRMS). One of the main features was a transient $^{13}C/^{12}C$ peak (2 h after intake) in nonesterified fatty acids bound to albumin with kinetics similar to that observed in triglyceride-rich lipoproteins. In contrast, a more progressive and sustained rise of ^{13}C-DHA could be observed in lysoPC also bound to albumin. Interestingly, the kinetics of ^{13}C-DHA accumulation into leukocyte and platelet phospholipids was much faster than in erythrocytes and fitted with the ^{13}C-DHA appearance in the nonesterified pool. In contrast, the ^{13}C-DHA accumulation in erythrocytes was coincident with the ^{13}C-DHA appearance in lysoPC (Brossard et al., 1997). Considering the accumulation of DHA in erythrocytes as a putative index of DHA accretion in the brain (Innis, 1992), we hypothesized that lysoPC might be a potential source of DHA for the brain. The rat studies reinforced this hypothesis. Indeed a single-dose intake of ^{13}C-DHA in triglycerides under the same conditions as in humans allowed us to confirm that the kinetics of ^{13}C-DHA in plasma lysoPC was similar to that in erythrocyte and brain phospholipids (Brossard et al., 1996).

In a second set of experiments, ^{13}C-DHA was ingested in phosphatidylcholine (^{13}C-DHA-PC) containing around 50% of DHA. This preparation was ingested by human volunteers under similar conditions as that of ^{13}C-DHA in triglycerides. The rationale of this second approach was to evaluate whether it would facilitate the availability of DHA in lysoPC and in turn potentiate its uptake by erythrocytes. The main results were the following: 1) the appearance of ^{13}C-DHA in plasma nonesterified FA was delayed (threefold) but not that in lysoPC; 2) the overall availability of ^{13}C-DHA in the whole blood was slightly decreased compared to the intake of ^{13}C-DHA-TG, but the rate of incorporation into erythrocytes was relatively higher; 3) both the lysoPC and nonesterified FA (to a lesser extent) contributed in the accretion of DHA in erythrocyte phospholipids, presumably explaining the relatively high incorporation in that pool as stated in 2) (Lemaitre-Delaunay et al., 1999). Assuming again that the incor-

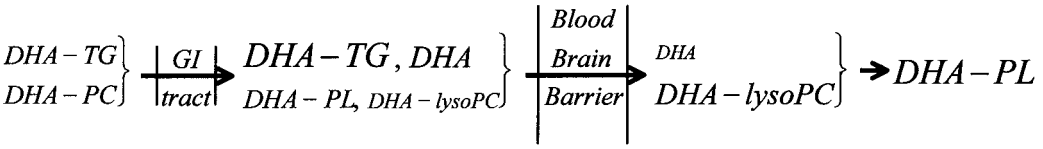

Fig. 1. This scheme summarizes the main data reported in the text. It means that DHA, ingested in triglycerides (DHA-TG) or phosphatidylcholines (DHA-PC), is available to the blood in various lipid forms, the major ones being triglycerides and phospholipids (DHA-PL) bound to lipoproteins, and the nonesterified pool (DHA) and lysoPC (DHA-lysoPC) bound to albumin. The BBB appears to preferentially take up DHA when it is esterified in lysoPC to ultimately accumulate it in brain phospholipids.

poration of DHA in erythrocytes may be an index of its accretion in the brain, we may conclude that the ingestion of DHA in PC may favor its bioavailability to the brain.

DHA Uptake by the Brain

Direct evidence for a preferential brain uptake of DHA from lysoPC as compared to nonesterified FA was obtained in rats and by using an in vitro model of blood brain barrier (BBB).

The uptake of various FA by the developing brain has been studied in 20-d-old rats. Each radiolabeled fatty acid was first incubated with albumin either in nonesterified form or esterified at the sn-2 position of lysoPC. Each preparation was then injected in the rat and the labeling was evaluated in different organs (Thiès et al., 1992, 1994). We found first that the higher the unsaturation of the FA studied, the more efficient was its brain uptake, especially when the FA was esterified in lysoPC. Also the rate of incorporation of unsaturated FA was much higher when esterified in lysoPC compared to the nonesterified form. As a matter of fact, AA was sixfold more actively taken up from lysoPC than from nonesterified AA (Thiès et al., 1992). Further studies with DHA showed that its lysoPC form was preferred by 12-fold when compared to nonesterified DHA (Thiès et al., 1994). This preference, observed for the uptake of DHA by the brain, did not occur in other organs considered. In the heart, kidney, and liver the preferential form was instead the nonesterified DHA (Thiès et al., 1994). This indicates that lysoPC might be a privileged and specific carrier of DHA, as well as AA to a lesser extent, to the brain.

In order to investigate the mechanism by which such a preferential uptake could occur, a model of reconstituted BBB was used. This model, consisting of cocultured brain-capillary endothelial cells and astrocytes, was reported to reasonably mimic the in vivo BBB (Meresse et al., 1989; Dehouck et al., 1992). The crossing of the reconstituted BBB by DHA was found highest when it was esterified in lysoPC compared to the nonesterified form, but this preference required the co-culture of the two cell types. Indeed, the absence of astrocytes or of the culture medium of both types of cells prevented the endothelial-cell monolayer to be preferentially crossed by DHA esterified in lysoPC as compared to nonesterified DHA (Bernoud et al., 1999). On that basis, the fatty acid form seems to be crucial for the observed preference rather than the fatty acid nature, as palmitic acid was also much better transferred through the endothelial-cell monolayer when esterified in lysoPC than when it was in its nonesterified form (Bernoud et al., 1999). This suggests that the BBB contains a facilitation system for the transfer of lysoPC from the blood to the brain.

Conclusion

Considering albumin as the physiological vehicle of fatty acids to target tissues, it has been found that PUFA are present in the nonesterified and lysoPC pools in similar amounts. Moreover, it appears that the latter pool might be preferred to the former for the PUFA accretion in the brain. Figure 1 summarizes the metabolic fate of DHA provided by food and preferentially taken up by the brain.

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