DHA involvement in neurotransmission process

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Abstract: *The very high enrichment of the nervous system in the polyunsaturated fatty acids, arachidonic (AA, 20: 4n-6) and docosahexaenoic acids (DHA, 22: 6n-3), is dependant of the dietary availability of their respective precursors, linoleic (18: 2n-6) and α-linolenic acids (18: 3n-3). Inadequate amounts of DHA in brain membranes have been linked to a wide variety of abnormalities ranging from visual acuity and learning irregularities, to psychopathologies. However, the molecular mechanisms involved remain unknown. Several years ago, we hypothesized that a modification of DHA contents of neuronal membranes by dietary modulation could change the neurotransmission function and then underlie inappropriate behavioural response. We showed that, in parallel to a severe loss of brain DHA concomitant to a compensatory substitution by 22:5n-6, the dietary lack of α-linolenic acid during development induced important changes in the release of neurotransmitters (dopamine, serotonin, acetylcholine) in cerebral areas specifically involved in learning, memory and reward processes. Data suggested alteration of presynaptic storage process and dysregulations of reciprocal functional interactions between monoaminergic and cholinergic pathways. Moreover, we showed that recovery of these neurochemical changes was possible when the deficient diet was switched to a diet balanced in n-3 and n-6 PUFA before weaning. The next step is to understand the mechanism involved. Particularly, we focus on the study of the metabolic cooperation between the endothelial cell, the astrocyte and the neuron which regulate synaptic transmission.These works could contribute to the understanding of the link between some neuropsychiatric disorders and the metabolism of n-3 PUFA, through their action on neurotransmission.*

Key words: *acetylcholine, behaviour, brain, DHA, dopamine, central nervous system, serotonin (5-HT)*

Introduction

The mammalian brain is particularly rich in polyunsaturated fatty acids (PUFA), mainly arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:5n-6) for the n-6 and the n-3 series, respectively. The brain contents of these PUFA are greatly affected by feeding of oils containing their respective precursor, linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3). Moreover, DHA could be obtained directly from aquatic sources.

The accumulation of DHA in brain membranes is particularly high during the perinatal period, coinciding with the formation of synapses [1, 2]. Thus, Cunnane *et al.* [3] have shown that during the first 6 months of life of a human infant, the brain accumulates around 5 mg of DHA every day, which represents half of the daily accumulation in the entire body. Then, at the adulthood, DHA can reach half of the quantity of PUFA inserted into the phospholipids that build the structure of neuronal membranes.

In addition to this structural function, DHA plays an important role in cell signaling, as a precursor of molecules such as docosanoids [4, 5]. It also regulates the expression of many genes, some of which are involved in the process of synaptic transmission [6]. Thus, studies using DNA microarrays suggested that n-3 PUFA regulate the expression of genes involved in cerebral energetic metabolim (cytochrome c oxydase), synaptic plasticity (α-synuclein), signal transduction (calmodulin I), neurogenesis or yet cytoskeleton formation and neurotransmission process [7-11].

n-3 PUFA and behaviour

For the last decades, many studies have revealed the role of n-3 PUFA, and more particularly DHA, in behaviour and learning. Thus, rodents subjected to a diet deficient in DHA, or in its precursor, showed reduced attention, modifications of habituation, anxiety and locomotor response to novelty [12-16]. They also exhibited an aggressive behaviour and increased depression symptoms in the forced swim test [17]. The rodents' learning ability was also impaired, and their performance in spatial memory and discrimination tasks was lower [18-20]. In addition, n-3 PUFA-deficient animals were more resistant to extinction, suggesting a reduced behavioural flexibility [21]. However, most of these effects were reversed by a dietary supplementation with long chain n-3 PUFA [22-24].

These different kinds of behaviours are underlain by different neuronal pathways that exert a reciprocal presynaptic control in specific cerebral areas.

Neurotransmission is a fundamental function

In the brain, neurotransmission is a fundamental function that allows a chemical communication between the different types of neural cells, through the release of neurotransmitters by exocytosis process. This process requires the fusion of the vesicular and the neuronal membranes. Therefore, it strongly involves phospholipids and fatty acids. It may not be a coincidence that biological membranes that are naturally enriched in DHA, such as neurons, rod outer segments and sperms, are predisposed to undergo vesicle formation and fusion. In addition, exocytosis involves a number of specialized proteins involved in targeting vesicles to the active zone and in the mediation of vesicle fusion with the presynaptic membrane, and some of them have been described to be located in lipid rafts [25, 26]. Therefore, the lipid environment, and more particularly the DHA levels in membranes, is likely to influence the efficiency of the exocytosis.

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In this context, we stated the hypothesis that the dietary modulation of the DHA content of the brain could change the neurotransmission function and then underlie the impairments of cognitive processes. In particular, we focussed on the release of dopamine in basal ganglia, for its key role in locomotion, reward and motivation, of serotonin in the frontal cortex, involved in anxiety and of acetylcholine in the septo-hippocampal pathway, for its crucial role in learning and memory.

Effects of dietary n-3 PUFA deficiency on brain phospholipid membrane composition

The most widely used method to study the function of DHA in the nervous tissue is to induce its deficiency in the brain from the lack of α-linolenic acid in the diet, during the gestation and lactation periods, throughout one or several generations of offspring.

We provided F2 rats with a diet free of rapeseed oil, compared to rats fed a control diet containing rapeseed and peanut oils. This leads to a severe decrease in DHA levels, notably in the phosphatidylethanolamine (PE) of the frontal cortex, with a well-established compensatory substitution by n-6 PUFA (mainly 22:5n-6, DPA), which are normally absent from brain membranes and thus signs the status of deficiency in the rats [27]. However, we showed that the range of variations of DPA and DHA levels following an α-linolenic acid deprivation were not identical in every sub brain regions. Thus, the most dramatic reduction of the DHA level takes place in the frontal cortex, as compared to striatum, hippocampus and septum. Very recently, Levant *et al.* [28] showed a depletion of brain DHA content in female undergoing pregnancy and lactation; this depletion was the greatest in the frontal cortex compared to seven other cerebral structures. Inversely, Carrié and collaborators [22] have shown that supplementation of deficient mice with phospholipids enriched in DHA restored normal fatty acid composition in all brain regions, except for frontal cortex. These data illustrated the differences in avidity for DHA between the cerebral areas previously described [29]. Therefore it seems that all of the cerebral areas do not respond identically to the lack of DHA precursor; and this may be in relation with functional specificity.

Effects of diet-induced decrease in brain DHA on neurotransmission function

Dysfunction of the dopaminergic mesocorticolimbic loop

We studied the dopaminergic system by measuring the levels of dopamine and their metabolites (DOPAC and HVA) in the frontal cortex. The results showed an important reduction of the neurotransmitter levels in deficient rats *(figure 1)*, whereas the turnover of DOPAC and HVA increased *(figures 2A and 2B)* [30, 31]. Moreover, we subsequently showed that the cortical release of dopamine after inhibition of the noradrenaline and dopamine uptakes (by inhibition of the transporters) was unchanged in deficient rats, whereas, as expected, it increased in the synaptic cleft of control rats *(figure 3)*.

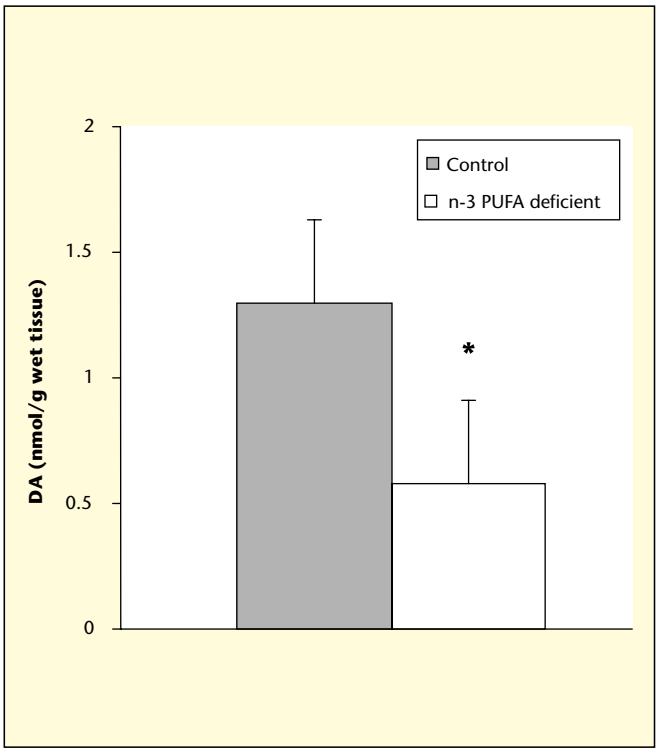

Figure 1. *Dopamine levels in the frontal cortex of 2-months-old rats fed a diet deficient in alpha-linolenic acid or a control diet (nmol/g wet tissue; mean ± SD; n = 8). *Significantly different between dietary groups (p < 0.05, ANOVA) [30].*

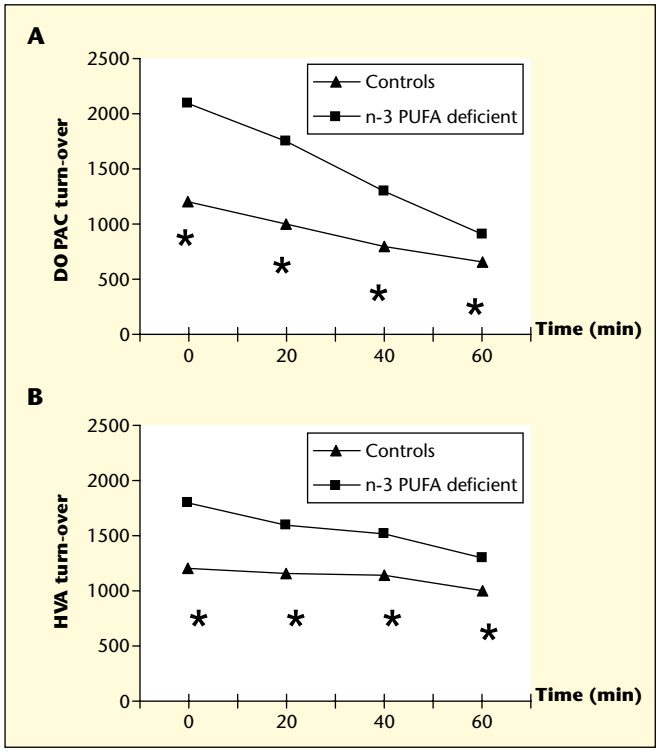

Figure 2. *Turn-over of DOPAC (A) and HVA (B) measured by microdialysis in the frontal cortex of 2-months-old rats fed a diet deficient in alpha-linolenic acid or a control diet (fmol/100 μL dialysate; mean ± SD; n = 8). *Significantly different between dietary groups (p < 0.05, ANOVA) [31].*

These data suggested that the process of presynaptic dopamine storage could be altered in deficient animals. To support this hypothesis, we looked at the vesicular stage, using dual-probe microdialysis to monitor the release of dopamine from the vesicular pool by tyramine stimulation in the frontal cortex and in the nucleus accumbens, both structures being involved in reward and learning process. The results showed that the response to tyramine was significantly reduced in deficient rats, by 70% in the frontal cortex *(figure 4A)* and by 90% in the nucleus accumbens *(figure 4B)* [32-34]. However, this effect was abolished by a resepine pretreatment, which depletes the dopamine vesicular store, showing a reduced dopamine reserve in the presynaptic vesicles. These results have been confirmed by the study of the density of dopamine vesicles, using immunolabeling with a dopamine antibody and in situ hybridization of vesicular transporters [31, 33, 34].

All this led to the conclusion that the dopamine vesicle compartment was reduced by 30% in the frontal cortex of deficient rats, resulting in a reduced cortical inhibition on the ventral parts, particularly on the nucleus accumbens. Moreover, the mRNA expression of D2 receptors was 30% lower in the frontal cortex and 20% higher in the nucleus accumbens of deficient rats [31, 33].

Moreover, Kuperstein *et al.* [11] has studied in detail the consequences of DHA deprivation in rats during the early development on the expression of a battery of genes incoding neurotransmitter receptors. In particular, they showed a remarkable elevation of the dopamine D1 and D2 receptors genes, in discrete regions of the mesolimbic and mesocortical pathways, notably the nucleus accumbens, the prefrontal cortex and the hippocampus, or yet in the ventral tegmental area. The authors attributed this over-expression to a compensatory mechanism resulting from the possible impairment of the dopamine synthesis, storage or processing, in order to enable the targeted synapses to act even with low levels of DHA.

To sum up, all these data suggest that an inadequate intake of DHA results in a dysfunction of the dopamine mesocorticolimbic loop, leading to an hypodopaminergia in the cortical areas, responsible for inattention, and to an hyperdopaminergia in the nucleus accumbens, responsible for hyperactivity and for an inefficient reward process. The reduction of dopamine reserves can then be related to the inappropriate behavioural response previously observed in deficient animals, since they may now not be sufficient to achieve a high release during stimulated cognitive processes.

Alteration of dopamine-related behaviour

Interestingly, a lower dopaminergic activity in the frontal cortex associated to a higher one in the nucleus accumbens, as we observed in the deficient rats, is considered to be a biological substrate of sensation-seeking that could induce locomotor hyperactivity [35, 36]. To explore this relationship, we measured the

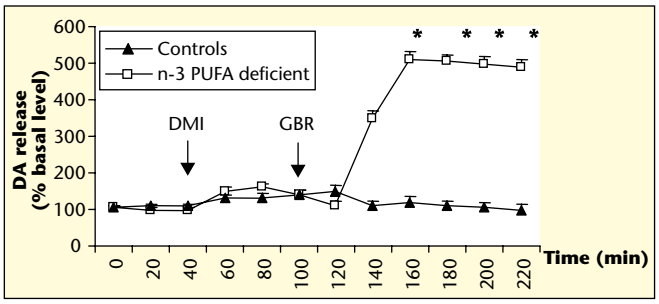

Figure 3. *Effect of blockage of noradrenaline- (DMI, 20 mg/kg i.p.) and dopamine-uptakes (GBR 12909, 20 mg/kg, i.p.) on the release of dopamine in the frontal cortex of 2-months-old rats fed a diet deficient in alpha-linolenic acid or a control diet (% basal level; mean ± SD; n = 8). *Significantly different from basal level (p < 0.05, ANOVA) [31].*

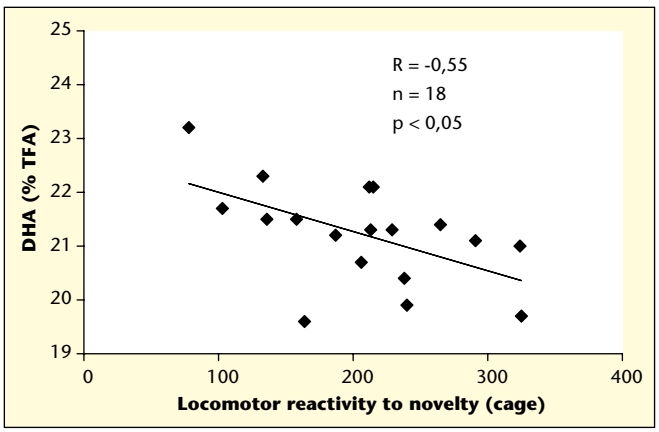

Figure 5. *Correlations between DHA contents of phosphatidylethanolamine (PE) expressed as % of total fatty acids (% TFA) in frontal cortex and activities in response to novelty measured in a cage for 1 hour. Values of Bravais-Pearson's correlation test are indicated [37].*

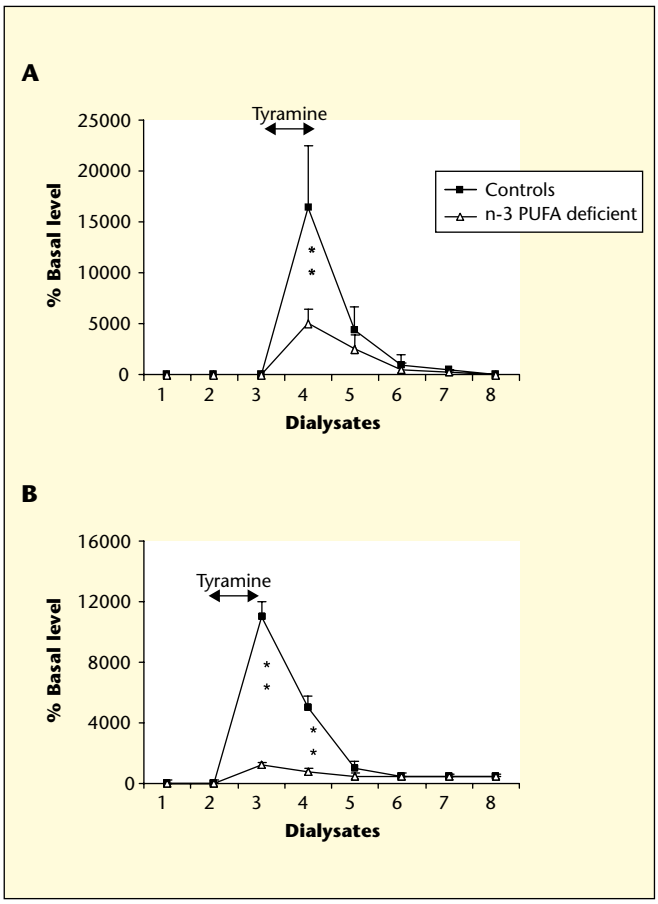

Figure 4. *Dopamine release under tyramine stimulation (200 μM, in situ perfusion) in the frontal cortex (A) and in the nucleus accumbens (B) of 2-months-old rats fed a diet deficient in alpha-linolenic acid or a control diet (% basal level; mean ± SD; n = 8). **Significantly different between dietary groups (p < 0.01, ANOVA) [32, 33].*

lomotor activity in response to novelty in a population of rats placed in a cage of activity for 1 hour. Despite all these rats fed the same standard lab diet, we observed an inverse relationship between the DHA levels in PE of the frontal cortex and the general motor activity: hyperactive individuals having less DHA than hypoactive ones *(figure 5)* [37]. When we compared rats fed a balanced diet and rats fed an α-linolenic acid deficient diet from conception, we saw that the last exhibited a severe hyperactivity in a circular corridor during the three consecutive days of test *(figure 6)* (unpublished results). Thus, the response to novelty was negatively linked to the DHA content of phospholipid membranes, whereas no association was found with n-6 PUFA.

Dysfunction of the hippocampal serotoninergic system (figure 7)

Using microdialysis, Kodas *et al.* [38] showed a dramatic increase in the basal synaptic release of serotonin in the hippocampus of adult awake rats fed an α-linolenic acid deficient diet, as compared to controls. Inversely, the release was reduced under pharmacological stimulation. The authors also studied the recovery of the serotonin release after the deficient diet was switched to an n-3 PUFA-adequate diet, at different stages of the neurodevelopment (at birth, 7 or 14 days postnatal, or at weaning). The results showed that, when given during the lactation period, the adequate diet restored both the fatty acid composition of the brain and the serotonin release. Whereas after weaning, the adequate diet did not allow any recovery for 5-HT, despite a normalization of DHA levels in the hippocampal membranes.

Dysfunction of the septo-hippocampal cholinergic system

The cholinergic system was investigated in accordance with its critical role in the processes underlying arousal, attention, learning and memory [39-41]. In order to check the proposal of an alteration of the cholinergic system under DHA-deficient conditions, we looked at the release of acetylcholine in hippocampal and cortical synapses of rats fed with diets containing different amounts of DHA (0 to 300 mg DHA/100 g diet) supplied by egg-phospholipids.

We showed that first, at rest, the synaptic release of acetylcholine was increased by 70% in the hippocampus of α-linolenic acid-deficient animals as compared to controls receiving adequate levels of n-3 and n-6 PUFA. But, the maximum KCl-stimulated release was reduced by 30%, associated to a 70% loss of DHA in phospholipid membranes *(figure 8)* [27]. Secondly, we showed that a supply of 200 mg of DHA/100 g diet is needed to ensure a release of acetylcholine and an incorporation of DHA in the membranes equivalent to those of the control rats *(figure 9)* [42]. These modifications were not related to changes in the catabolic acetylcholinesterase and choline uptake activities, nor in the density of the vesicular acetylcholine transporter.

Conclusion

These data show that the variation of the DHA contents in brain phospholipid membranes is associated with the modification of several neurotransmission systems, and more particularly with changes in the release of neurotransmitters. Specific changes in dopamine release in the frontal cortex and in the nucleus accumbens seem to be linked, probably through anatomo-functional interactions between the 2 areas. This can be connected to the effect of a DHA deficit on the impairment of attention and of the reward process, that contribute to the damaging of learning and to the slowing of extinction. Moreover, the basal and the stimulated releases were inversely affected for acetylcholine and 5-HT in the hippocampus; this could be involved in reduced spatial learning and increased anxiety in deficient animals.

It has been shown that these neurochemical changes could potentially be reversed by an adequate diet, depending on when the intervention occurs. In particular, weaning seems to be a pivotal period after that all recovery was impossible.

However, it must be kept in mind that these different neuronal pathways exert reciprocal presynaptic control in specific cerebral areas to regulate the behavioural response.

We have now to understand what is the mechanism involved in the modulation of the neurotransmission function depending on the presence or not of DHA in brain phospholipids.

The regulation of the synaptic transmission is the result of a complex metabolic cooperation between three intimate partners: the endothelial cell for the energy supply, the astrocyte network for the regulation of functional coordination between cells and plasticity, and the presynaptic neuron for the release of neurotransmitters.

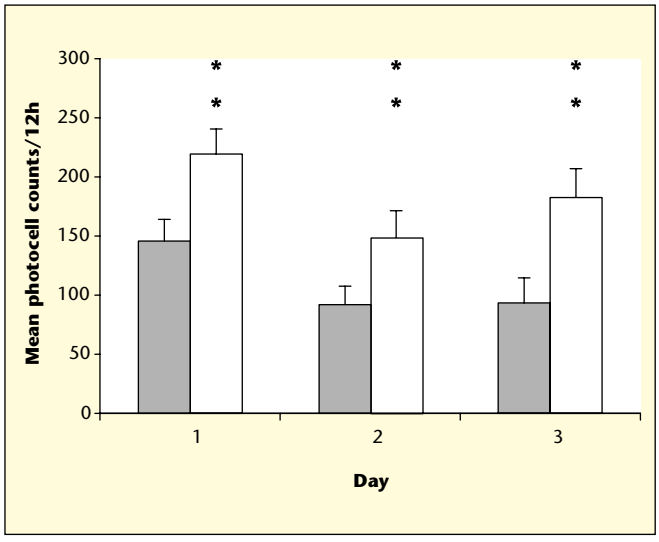

Figure 6. *Locomotor activity measured in a circular corridor for 12h-light for 3 consecutive days of 2-months-old rats fed a diet deficient in alpha-linolenic acid (white bars) or a control diet (black bars) (mean ± SD; n = 12). **Significantly different between dietary groups (p < 0.01, ANOVA).*

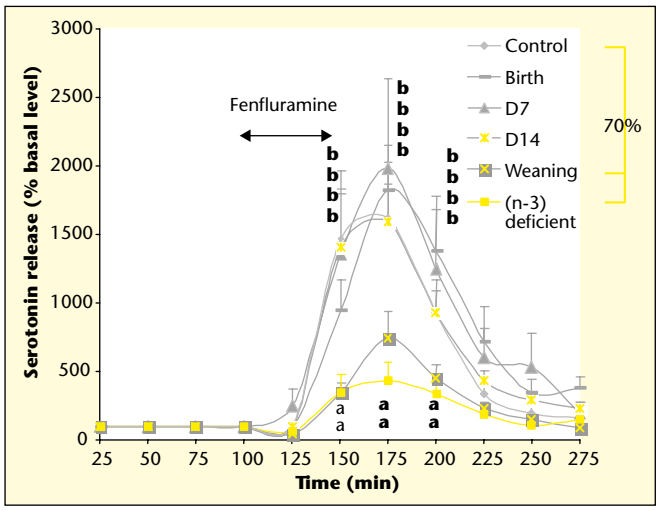

Figure 7. *Serotonin release under fenfluramine stimulation (1.25 mM, in situ perfusion) in the hippocampus of control, deficient or diet-reversed groups (7 days after birth, D7; 14 days after birth, D14, at weaning) of 2-months-old rats (% basal level; mean ± SD; n = 8). (a-b) Significantly different between dietary groups (p < 0.01, ANOVA) [38].*

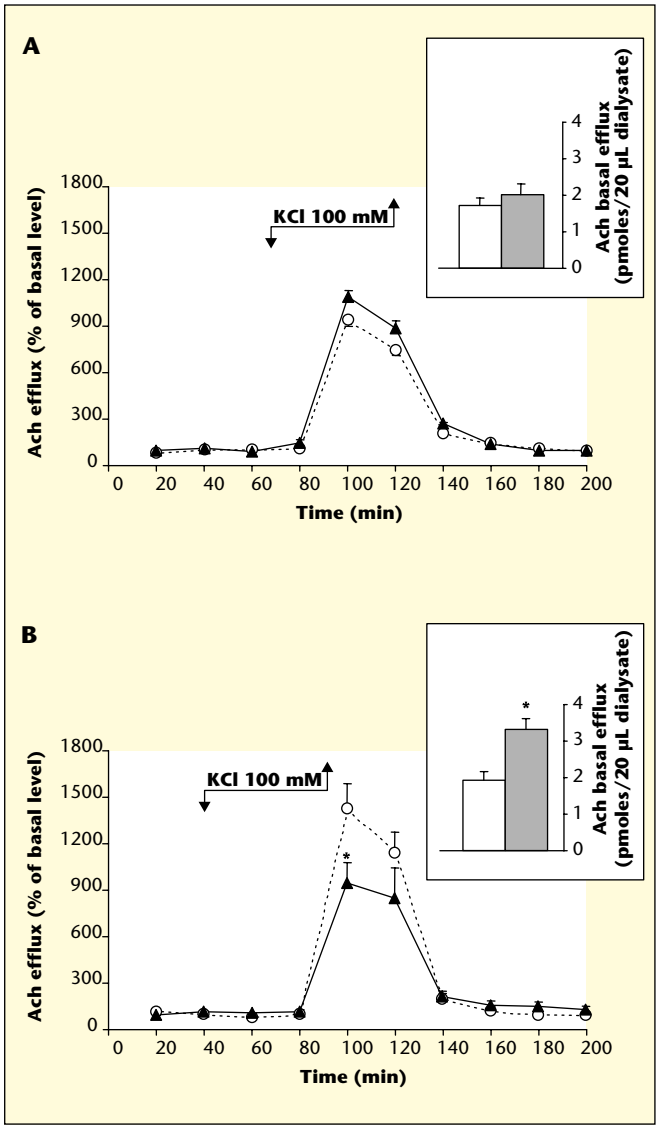

Figure 8. *Ach release in the frontal cortex (A) and the hippocampus (B) of 2-months-old control (open circles) and alpha-linolenic acid-deficient rats (black triangles) (% basal level; mean ± SEM; n = 6 to 9). *Significantly different between dietary groups (p < 0.01, ANOVA).* ***Insert****: basal Ach release in control (white bars) and alpha-linolenic acid-deficient rats (black bars). Values are expressed as the average (pmoles/20 µL of dialysate) of the three 20-minute dialysate collections immediately preceding 100 mM KCl perfusion. * Significantly different between dietary groups (Student's t-test; P < 0.05) [27].*

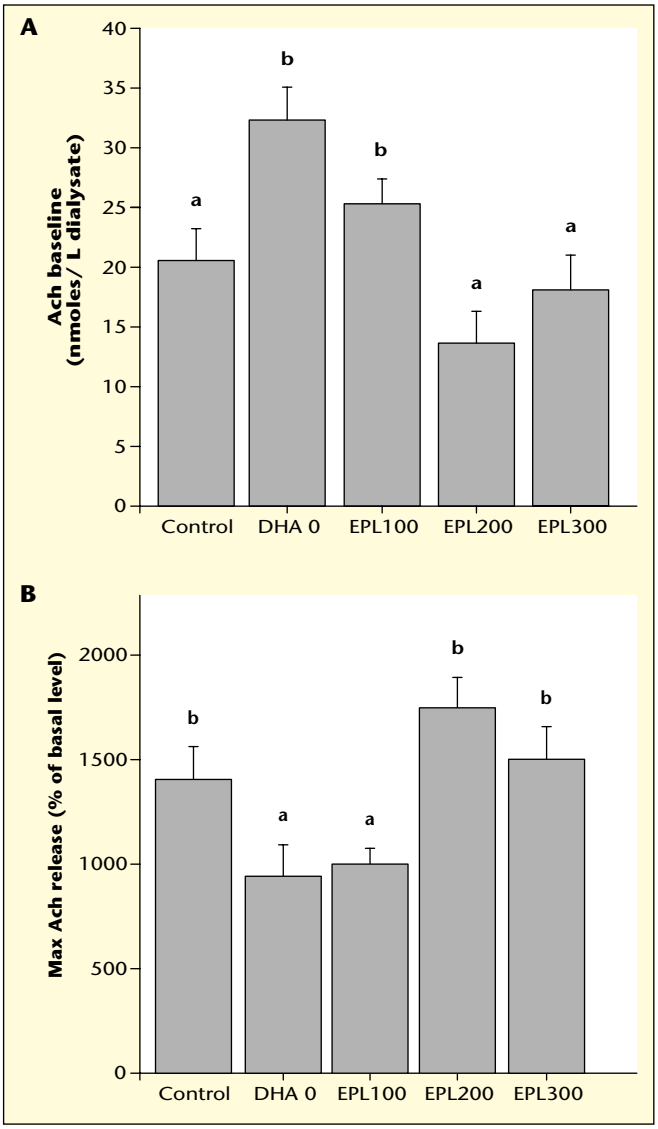

Figure 9. *Basal (A) and maximal release (KCl 100 mM, perfusion) (B) of Ach from the hippocampus of control (n = 9), n-3 PUFA deficient (DHA 0, n = 7), and egg phospholipids supplemented (EPL, mg DHA/100 g diet) (n = 5 to 8) 2-months-old rats (mean ± SD). Means without a common letter differ (P < 0.05, ANOVA) [42].*

We have shown that modifications of DHA levels in the phospholipid membranes of the three cell types led to changes in glucose transport [43] but also in gap junction coupling [44]. The hypothesis can be made that the proportions of DHA in membranes may have an impact on the morphological plasticity and on the different astrocyte functions involved in the regulation of synaptic transmission, and more particularly in the release of neurotransmitter in the synaptic cleft.

These data will help to understand the relationship between the n-3 PUFA metabolism and some neuropsychiatric disorders, such as depression or schizophrenia, in which altered neurotransmission systems are well known today. Thus, a PUFA imbalance could contribute to the predisposition to central nervous system pathologies by acting on the regulation of the neurotransmission function. This highlights the importance of optimal nutritional n-3 PUFA supply to prevent or at least buffer impairments of brain functioning.

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