Dietary oleic acid not used during brain development and in adult in rat, in contrast with sciatic nerve

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Abstract

In order to determine exactly the effect on the nervous system of concentration of dietary oleic acid on the fatty acid composition of different part of the nervous system, triglycerides were synthesized using chemical and enzymological methods. The dose-effect was determined using an experimental protocol with seven groups of rats who received a diet in which the oleic acid level varied from 0 to 6000 mg per 100 g diet, but the other ingredients were identical (in particular the essential fatty acids, linoleic and alpha-linolenic acid). Rats were fed the diets from two weeks before mating, and their pups were sacrificed aged either 21 or 60 days. When the level of oleic acid in the diet was increased, the main modifications observed in 21-day-old deficient animals were as follows. (i) For 18:1(n-9), in liver, plateau was reached at about 4 g oleic acid per 100 g diet. Below this level, the higher the dose the greater the response. In whole brain, brain myelin, and nerve endings (but not sciatic nerve) the oleic acid level remained optimal and constant whatever the level of oleic acid in the diet. (ii) 16:1(n-7) concentration decreased in liver and in sciatic nerve, but not in nervous tissue. (iii) In 60-day-old animals, results were generally similar to those in 21-day-old animals.

Keywords: Oleic acid; Dose-effect; Liver; Brain; Sciatic nerve; Brain myelin; Nerve endings

Oleic role in nervous tissue seems to be peculiar. Interestingly, neuronal differentiation is triggered by oleic acid synthesised and released by astrocytes [27]; albumin, a serum protein present in the developing brain, stimulates the synthesis of oleic acid by astrocytes, which promotes neuronal differentiation [28]. A functional link may exit between the effects of fatty acids (including oleic acid) on hypertension and the modulation of aminopeptidase activity by these compounds in rat astrocytes, as an example of target cell type in the central nervous system [24].

Adaptation to high-fat diet selectively reduces vagal and enteric neuronal sensitivity to intestinal oleate and suggests that reduced sensitivity to the satiation and gastric inhibitory effects of oleate in high-fat fed rats may be mediated by a selective reduction in the neuronal response to intestinal stimulation by fatty acid [12]. The hypothalamus and other regions within the central nervous system link the sensing of nutrients to the control of metabolism and feeding behaviour; intracerebroventricular administration of oleic acid markedly inhibits glucose production and food intake; the anorectic effect of oleic acid is independent of leptin and is accompanied by a decrease in the hypothalamic expression of neuropeptide Y [22]. Brain and hippocampus fatty acid composition in phospholipid classes of aged-relative cognitive deficit rats involve changes in oleic acid amount, notably in sphingomyelin [29].

A derivative of oleic acid, oleamide (a natural analogue of the endogenous cannabinoid anandamide), present hypogenic effect not related to changes in blood pressure, heart rate or body temperature [18]. Moreover, oleamide is a lipid mediator involved in the peripheral regulation of feeding [26].

The amounts of alpha-linolenic and linoleic acids that need to be supplied in the diet to permit elaboration of membrane structures in different organs, especially brain, have been determined [6]. The amounts needed to maintain the structures in place, that is to say ensure their renewal, has also been determined [4]. These studies have led to progress in commercial products: composition of vegetable oil mixtures, reformulation of formula milks. The logical consequence of these results, and in view of the composition of the main commercial vegetable oils, is that it has become indispensable to determine whether dietary oleic acid affects...
the metabolism and incorporation of oleic acid in the membranes.

In a previous study [5], we have shown that at the end of the gestation-lactation period in 21-day-old rats, dietary deficiency of oleic acid leads to a decrease in this acid (18:1(n-9)) in certain tissues (liver, kidney, testis, muscle, sciatic nerve), but not in others (heart, brain, myelin nerve endings). It is thus evident that the organism (in particular the liver) does not have sufficient oleic acid synthesizing capability to ensure a normal composition of the membranes of some organs. Thus, including for oleic acid, the conditional nature of the dietary need for polyunsaturates must be re-examined: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or 'conditionally-dispensable' fatty acids [14] is interesting for oleic acid.

Since it has been shown that the organism cannot synthesize (presumably by desaturation of stearic acid) all the oleic acid that it requires, the aim of this study was to determine the minimum dietary intake of oleic acid that is indispensable for brain development, if any.

Since commercial vegetable oils could not be used because they all contain oleic acid, triglycerides containing either oleic acid, alpha-linolenic acid, or linoleic acid were synthesized as previously described [5]. The diets contained linoleic or alpha-linolenic polyunsaturated fatty acids (in the proportion of 6:1, according to recommendations and our previous results) and triglycerides formed from oleic acid or stearic acid. Seven groups of rats received a diet whose essential fatty acid content, i.e. linoleic and alpha-linolenic acids, was identical, but in which the level of oleic acid varied from 0 to 6000 mg per 100 g diet. Three weeks before mating, seven groups of females each received one of the diets. Their male pups were sacrificed by decapitation aged 21 days (weaning) in one group of animals, and aged 60 days in another. For the whole brain and liver, values were the mean of at least seven different rats, from at least three different litters. For myelin and nerve endings, each value was the mean of at least four different preparations; each density gradient required at least four rats; thus, each value represents at least 16 rats from at least three different litters. Fatty acid profiles were obtained in whole brain, myelin, nerve endings (synaptosomes), sciatic nerve, and in liver. Lipids were extracted by the chloroform-methanol method according to Folch; methyl esters were obtained according to Morrission using methanolic BF3; fatty acids methyl esters were analyzed by gas chromatography using a capillary column. All techniques are routine in our laboratory and have been published. Experimental protocols were approved and comply with government directives (Ministry of Agriculture authorization No. 03007, of 4 June 1991). Statistical analysis was performed using Student’s t-test and ANOVA.

Table 1 summarizes, in 21-day-old animals, the main variations in fatty acid levels by comparing results for animals receiving the diet deficient in oleic acid (0 g/100 g) with those receiving the diet containing the highest level of oleic acid (6 g/100 g).

In 21-day-old animals, concerning 18:1(n-9), Fig. 1 shows that in liver and in sciatic nerve, plateau is reached at about 4 g of oleic acid per 100 g of diet. Below this level, the higher the dose the greater the response. From 0 to 6 g/100 g of oleic acid in the diet, 18:1(n-9) increases by 97.8%, in liver and 52.6% in sciatic nerve. In whole brain, brain myelin, and nerve endings, the oleic acid level remains optimal and constant whatever the level of oleic acid in the diet.

Concerning 16:1(n-7), Fig. 2 shows that the concentration of 16:1(n-7) decreases by 56.6% in the liver and by 46.6% in sciatic nerve, when dietary oleic acid increases from 0 to 6 g/100 g of diet. At higher levels, there is a plateau. No changes is measured in the level of 16:1(n-7) in whole brain, brain myelin, or nerve endings.

The concentration of 18:1(n-7) decreases in liver, (31.8%) when dietary oleic acid increases from 0 to 6 g/100 g; it stabilizes around 3–4 g/100 g diet. In the nervous system (including sciatic nerve), 18:1(n-7) is not significantly modified when the oleic acid level increases in the diet (data not shown).

An increase in dietary oleic acid induces a decrease in palmitic acid (16:0) in liver (22.6%) and in sciatic nerve (27.8%); the concentration of palmitic acid is not significantly modified in the nervous system when oleic acid is increased in the diet; in sciatic nerve the decrease is 28% (data not shown). The increase in dietary oleic acid does not significantly affect stearic acid (18:0) concentration in liver; results are similar in the nervous system, where changes are not significant except in sciatic nerve in which stearic acid decreases by 12.6% (data not shown).

Table 1

<table>
<thead>
<tr>
<th>Fatty acids g/100 g</th>
<th>18:1-n-9</th>
<th>16:1-n-7</th>
<th>18:1-n-7</th>
<th>16:0</th>
<th>18:0</th>
<th>∑(n-6)</th>
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<tr>
<td></td>
<td>0 6 %</td>
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<tr>
<td>Liver</td>
<td>18.6 36.8 97.8</td>
<td>7.6 3.3 56.6</td>
<td>4.4 3.0 31.8</td>
<td>29.7 22.9 22.9</td>
<td>11.0 9.4 18.9 15.8 16.4</td>
<td></td>
</tr>
<tr>
<td>Whole brain</td>
<td>14.2 15.0 - 0.8 0.8 - 3.2 2.9 - 25.1 24.5 - 21.7 21.5 - 17.8 17.6 -</td>
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<tr>
<td>Sciatic nerve</td>
<td>26.2 40.0 52.6 3.0 1.6 - 46.6 2.9 2.7 - 28.7 20.7 - 27.8 7.1 6.2 - 12.6 12.1 8.2 - 32.3</td>
<td></td>
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<tr>
<td>Synaptosomes</td>
<td>12.9 13.2 - 0.9 0.9 - 4.3 3.8 - 23.0 22.7 - 23.1 22.6 - 20.2 19.3 -</td>
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<tr>
<td>Brain myelin</td>
<td>27.1 25.9 - 0.6 0.7 - 4.4 4.9 - 13.8 13.6 - 21.6 22.2 - 11.8 12.0 -</td>
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a Twenty-one-day-old animals. All data are significant (P < 0.01).
The sum of (n-6) fatty acids is decreased by 16.4% in liver and 32.3% in sciatic nerve; in the nervous system, the sum of (n-6) fatty acids is stable in brain, myelin, and nerve endings. In general (results not shown), the concentration of 22:5(n-6) is constant in all organs and subcellular fractions, whatever the dietary level of oleic acid. However, concentrations are very low (less than 2%) in liver, whole brain, sciatic nerve, nerve endings, and brain myelin.

An increase in the level of dietary oleic acid does not alter the sum of (n-3) fatty acids (results not shown) in either the liver or the nervous system.

Effect of increasing doses of oleic acid in 60-day-old rats are shown in Figs. 3 and 4. In general, notably for 16:0, 18:1(n-9), 16:1(n-7), and 18:1(n-7), results are similar to those in 21-day-old animals, but with some differences, in particular a slight decrease in the level of oleic acid in liver (but not in brain tissue or sciatic nerve) at the highest dose of oleic acid in the diet.

In agreement with the dietary origin of liver oleic acid (this study), desaturation of stearate is not sufficient to increase the oleic acid concentration in cultured hepatocytes [23]. Thus, at least for liver, oleic acid is conditionally essential [5], the proposal to reclassify ‘essential fatty acids’ as ‘conditionally-indispensable’ or ‘conditionally-dispensable’ fatty acids [14] is indeed very interesting.

The fact that increasing levels of oleic acid in the diet do not modify the level of oleic acid in cerebral structures suggests two hypotheses. (i) First, the central nervous system has priority for the uptake of oleic acid, which raises the possibility of specific transport mechanisms at the blood–brain barrier. This hypothesis could be excluded as deuterated dietary oleic acid is not recovered intact in the brain, which seems to exclude a dietary origin [15]; moreover, during the period with a very high requirement it appears that the expression of genes involved in stearoyl-desaturase is independent of the myelinating signal in mouse central nervous system [16]. (ii) Second, the nervous system can synthesize all the oleic acid it needs, independently of its presence in the diet. In fact, the brain [9], like the peripheral nervous system [17], synthesizes oleic acid from stearic acid, stearoyl-CoA synthesis being present in sciatic nerve microsomes [2]. Acylation mechanism in sciatic nerve seems to involve an acyl exchange between oleyl-CoA and phosphatidylcholine [20]. Indeed, phospholipid acylation by mouse sciatic nerve microsomes does exist [11] and biosynthesis of very long chain fatty acids have been demonstrated in the sciatic nerve of the rabbit [10].

The origin of the precursor of oleic acid remains to be determined, as well as the mechanisms of synthesis and regulation. Stearic acid is actively synthesized by the brain [1,3], but it can be of exogenous origin, and pass through the blood–brain barrier [7,21]. Moreover, imaging of the passage of saturated fatty acids across the blood–brain barrier is used for examining signal transduction and neuroplasticity involving phospholipids [25].

In contrast, in minced tissue suspension, oleic acid incorporation into neutral lipids and arachidonic acid incorporation into PC, PE and phosphatidylinositol were increased in aged rats with respect to adult rats. This shows the ability and avidity of aged brain tissue in vitro to incorporate unsaturated fatty acids when they are supplied exogenously [19].

It should be noted that oleic acid deficiency results in few or no changes in levels of polyunsaturated fatty acids. This dose-effect study, in which increasing amounts of oleic acid were given to different groups of animals, showed that the minimum amount needed to stabilize this fatty acid in the organs was 4 g of oleic acid per 100 g of diet during the
gestation–lactation period in 21-day-old rats. In general, the same was true for 60-day-old animals. However, high doses of dietary oleic acid seem to induce a decrease in this fatty acid in liver in adult animal.

The relationships between the cellular and subcellular physiologies and the level of oleic acid in the membranes remain to be determined. Thus, taking into account the important physiologic role of oleic acid in brain, absence of exogenous supply seem to be normal. In contrast, exogenous very long chain saturated fatty acids are needed during brain development [8]. Application of new methods and analytical approaches to research on polyunsaturated fatty acid homeostasis are needed to elucidate quantitatively oleic origin in peripheral nervous system [13].

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