Biochimica et Biophysica Acta 1820 (2012) 1490–1495

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Conjugated linoleic acid in the maternal diet differentially enhances growth and cortical spreading depression in the rat progeny

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ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 22 May 2012
Accepted 23 May 2012
Available online 1 June 2012

Keywords:
Brain development
Brain electrophysiology
Conjugated linoleic acid
Physical parameter
Polyunsaturated fatty acid
Rats

ABSTRACT

Background: Conjugated linoleic acids (CLA) are fatty acids that are found in the lipids from goat milk, and appear to protect neurons from excitotoxicity.
Methods: We investigated in developing rats the effects of a maternal CLA-rich diet (containing 7% lipids from goat milk) on body development and cerebral electrical activity of the progeny from dams receiving the CLA diet during gestation (G), lactation (L) or both periods (G + L).
Results: Compared to a control group (C) receiving a diet with 7% soybean oil, body weight increased at 14, 21 and 28 days, but not at 35–45 days, in L and G + L groups ($P<0.05$). No intergroup difference was found on body and brain weights, body length, abdominal and thoracic circumferences, body mass index and abdominal to thoracic circumference ratio at 35–45 days. In contrast, at this later age the CSD velocities of propagation were significantly higher ($P<0.05$) in L as compared with the C and G group, and in the L + G, as compared with the C, G and L groups, suggesting a long-lasting brain effect.
Conclusion: These data indicate that a maternal CLA-rich diet can differentially influence body weight increment (short-term effect), and CSD propagation (long-term effect) in the progeny, and the lactation is the most critical period for such diet actions.
General significance: The facilitating effect of the lipids from goat milk on an excitability-related phenomenon in the brain (CSD) can be of clinical relevance, since CSD has been associated to neurological disturbances like migraine and epilepsy.

1. Introduction

Dietary lipids play an important role in the brain development. After the adipose tissue, the brain is the organ with the highest concentration of lipids [1]. In mammals, the dietary essential fatty acids (EFA) received by the pregnant and lactating mothers are extremely important for the brain development of their progeny, since the organism is not capable of synthesizing EFAs. Thus, the brain requirements of polyunsaturated fatty acids (PUFA) must be supplied by the diet [2]. Dietary deviations in maternal fatty acids intake throughout pregnancy may affect the nature of fetal fatty acids, affecting postnatal development.

Polyunsaturated fatty acids are synthesized in mammals from two precursors: α-linoleic acid (18:2*n*−6) and α-linolenic acid (18:3*n*−3), with the participation of the enzymes known as desaturases and elongases [3–6]. Strategies have been proposed to modify the maternal intake of certain essential fatty acids to warrant their availability to the fetus, but an excess of certain fatty acids may impair the availability of others, with undesirable consequences to the newborns [7]. One group of isomers of the linoleic acid with double bonds in *cis*-, *trans*- or mixed configurations, generically designated as "Conjugated linoleic acids" (CLA) [8], has been shown to have positive health effects such as attenuation of carcinogenesis and atherogenesis, and modulation of the immune system, and diabetes [9]. CLA comprise a family of isomers of linoleic acid (18:2n−6) that are formed by biohydrogenation in the rumen of ruminants by the action of rumen bacteria [10,11]. The goat milk is a good source of CLA. It is an alternative for infants and adults sensitive or allergic to cow milk [12]. The content of CLA in goat milk fat is influenced mainly by the diet composition. Large amounts of linolenic and linoleic acid in the goat diet result in elevated content of CLA in the milk [9,13].

Regarding the action of CLA on the central nervous system, it is known that CLA crosses the blood–brain barrier and is incorporated and metabolized in the brain [14]. The chronic dietary CLA intake can reduce cerebral prostaglandin E2 in the peripheral and central nervous system [15]. Besides that, CLA exerts antiangiogenic actions

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doi:10.1016/j.bbagen.2012.05.010

in the mammalian brain [16] and it has recently been shown to protect cortical neurons from excitotoxicity [17].

The influence of the dietary lipids on neuronal excitability has also been investigated using the electrophysiological phenomenon known as cortical spreading depression (CSD) [18,19]. However, no data are available yet on the excitability effects of CLA. This issue has been addressed in the present study by recording the excitability-related CSD phenomenon.

CSD is a fully reversible, excitability-related neural response first described as a slowly propagating wave of depression of spontaneous neuronal activity produced by electrical, mechanical or chemical stimulation of one point on brain tissue, from which it spreads concentrically to remote cortical regions [20]. CSD has already been demonstrated in the central nervous system of several laboratory animals [21] and also in the human brain [22]. The brain recovery process from CSD is completed 5–10 min after being elicited, rendering again the brain tissue prone to another CSD. Measuring CSD velocity of propagation along the cortical tissue is a reasonable and easy way of estimating the brain CSD susceptibility. During the initial phase of CSD, the spontaneous brain electrical activity is depressed and this EEG depression spreads slowly all over the brain cortical surface [20]. This phenomenon is reversible and is characterized by particular ionic, metabolic and hemodynamic changes [21]. CSD is dependent of the neuron–glia interactions and can be affected by several conditions including nutritional [23] and pharmacological [24] manipulations. There are several reports showing that CSD seems to be involved in various pathophysiological events with clinical importance for humans including ischemia [25] migraine [26], and epilepsy [27].

Using CSD as a neurophysiologic parameter, in this study we investigated the impact of feeding pregnant and/or lactating rat dams with a CLA-rich diet on the developmental (body weight and metric parameters) and electrophysiological CSD effects on their male progeny. We postulated that susceptibility of the brain to CSD could be altered in the pups by the maternal dietary CLA treatment. Two questions were addressed in the weaned progeny. First, how does administration of a CLA-rich diet to pregnant and/or lactating dams affect body growth and CSD propagation in the progeny? Second, in which of the treatment periods would the CLA-rich diet have more of an impact in terms of altering the susceptibility of the progeny brain to CSD?

2. Methods

2.1. Animals

Female Wistar rats (120–150 days of life; 300 ± 50 g) were mated in the proportion of two female to one male. Once the presence of vaginal sperm plug was detected (considered gestational day 0), the female was removed and housed in individual cages with free access to food and water. The animals were kept in a room with temperature of 22 ± 1 °C, a 12/12 h light/dark cycle (light phase beginning at 6:00 AM) and relative humidity of 65%. Litters were standardized to have six pups. The experimental procedures were approved by the Ethics Committee for Animal Research from the Universidade Federal de Pernambuco, which complies with the recommendations from the National Institute of Health (Baltimore, USA).

2.2. Maternal diets

Both the standard and the experimental diets were formulated based on recommendations of the American Institute of Nutrition (AIN-93G) [28], and manufactured by the company Rhoster (São Paulo/Brazil), being stored under refrigeration. The diets contained 7% fat from goat milk (experimental diet), or from soybean oil (standard diet), as the only fat source. Analysis of the fatty acid composition of each diet revealed differences in the saturated-to-polyunsaturated fatty acids ratio (Table 1). Moreover, the experimental diet contained 1.2% of CLA, whereas in the standard diet no CLA was detected. Therefore, both diets differed in lipid quality, but not in quantity.

Table 1
Fatty acid percent composition of the diets.

Fatty acid	Control diet	CLA diet
C4:0	–	0.95
C6:0	–	0.96
C8:0	–	0.99
C10:0	–	3.60
C12:0	–	2.10
C14:0	0.42	6.40
C15:0	–	0.63
C16:0	17.10	24.26
C16:1	–	0.34
C17:0	0.34	0.53
C18:0	5.93	20.94
C18:1(n9)	28.53	28.79
C18:2(n6)	42.56	5.97
C18:2(n6)CLA	–	1.20
C18:3(n3)	1.89	0.63
C20:1	2.18	–
C22:0	0.53	–
n6/n3	22.52	11.38
Others	0.52	1.71
Total (%)	100	100

Values are means of triplicate measurements.

One control- and three experimental groups were studied. The control group (C) received the standard diet. The three experimental groups received the experimental diet at three different periods: (1) during the gestation (group G); (2) during lactation (group L); (3) during gestation plus lactation (group G + L). After weaning all pups were kept on the control diet until the day of the CSD recording session.

2.3. Recording of cortical spreading depression

At 35–45 days of life, under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, i.p.), two trephine holes (3 mm in diameter) were drilled on the right parietal bone. The cortical spontaneous electrical activity (Electrocorticogram) and the slow potential change accompanying CSD were recorded simultaneously in these two points on the cortical surface during 4 h by using a pair of Ag–AgCl agar-Ringer electrodes. These electrodes consisted of 5-cm long plastic conic pipettes (0.5 mm tip inner diameter), filled with Ringer solution, solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. The pipettes were pair-wise fixed together with cyanoacrylate glue, so that the inter-electrode distance was kept constant for each pair of electrodes. A common reference electrode, of the same type, was placed on the nasal bones. Spreading depression was elicited at 20-min intervals, through a third hole (2 mm in diameter) drilled on the right frontal bone by applying, for 1 min, a small cotton pledget (1–2 mm in diameter) soaked with 2% KCl solution (approximately 270 mmol/L). The three holes were aligned in the anterior–posterior direction and parallel to the midline. The CSD propagation velocity was calculated based on the time required for a CSD wave to cross the distance between the two recording electrodes. The incidence of "multiple" CSD, i.e., two or more CSD episodes appearing after a single KCl stimulation, was also quantified. During the recording session, the rectal temperature was maintained at 37 ± 1 °C by means of a heating blanket.

2.4. Physical parameters

After the recording session, the still anesthetized animal was submitted to the analysis of the following physical parameters, as described by Novelli et al. [29]: body mass index (BMI; defined as weight [g]/length^2 [cm^2]), thoracic circumference (TC) [cm],

abdominal circumference (AC) [cm] and AC/TC ratio (cm). Finally, the anesthetized animal was killed by inserting a fine needle through the cisterna magna, provoking bulbar lesion with cardio-respiratory arrest. The brain was then removed and weighed.

2.5. Measurement of brain fatty acids

For identification and quantification of fatty acids in the total brain, the lipids from brain homogenates were separated, transmethylated and extracted in isooctane. The methyl fatty acids were analyzed by a gas chromatography apparatus with a fused silica capillary column of 100 m (SP 2500) with hydrogen as carrier gas (1.8 mL/min) and a flame ionization detector. Each sample was rotated to a temperature gradient from 70 to 240 °C to determine the peak identification of fatty acids.

2.6. Statistical analysis

Intergroup differences in weights, physical parameters and CSD velocity of propagation were analyzed by means of analysis of variance (ANOVA) followed by a post-hoc test (Tukey), when indicated. The incidence of "multiple" CSDs was analyzed by the Kruskall–Wallis ANOVA on ranks. Differences were considered significant when $P \leq 0.05$.

3. Results

3.1. Body and brain weights and physical parameters

As shown in Fig. 1, the mean ± sd body weights were higher in the groups G, L and G + L, as compared to the control group, and the differences were significant ($P<0.05$) at postnatal days 14, 21 and 28 (in this late age, only in groups L and G + L). The mean values (in g) for the control, G, L and G + L groups were respectively 27.55 ± 2.07, 31.89 ± 1.78, 31.38 ± 3.27 and 33.26 ± 4 on day 14; 39.53 ± 2.45, 51.19 ± 3.47, 50.60 ± 4.57 and 51.79 ± 8.60 on day 21; 69.51 ± 4.87, 78.90 ± 5.47, 80.63 ± 7.79, and 82.46 ± 13.24 on day 28; and 117.32 ± 7.68, 121.79 ± 10.95, 119.17 ± 14.67 and 125.02 ± 16.49 on day 35.

No intergroup statistical difference was found regarding the body length, abdominal circumference (AC), thoracic circumference (TC), body mass index (IMC), abdominal to thoracic circumference ratio (AC/TC) as well as brain weight measured on the day of the CSD recordings. The mean values for these physical parameters are shown in Table 2.

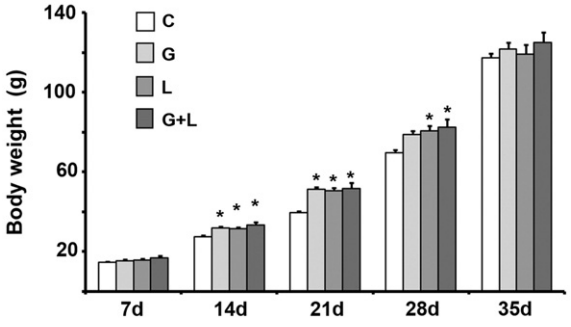

Fig. 1. Body weights (mean ± S.E.M.) of rats, evaluated at 7, 14, 21, 28 and 35 days of life. C, G, L and G + L are groups from progenies of dams that received respectively the control diet (C), or the CLA-diet during the gestation period (G), or during the lactation period (L), or during both gestation + lactation (G + L). The number of animals in each group is 10. The asterisks indicate values from the experimental groups that are significantly higher than the corresponding control values ($P<0.05$; ANOVA plus Tukey test).

3.2. CSD elicitation and propagation

As a rule, the 1-min topical application of 270 mmol/L KCl on the frontal cortex elicited a single CSD wave, which propagated and was recorded by the two electrodes located more posterior in the stimulated hemisphere. The ECoG depression and the slow potential recordings confirming the presence of CSD after KCl stimulation on the cortical surface of four rats (one from each of the four groups) are presented in Fig. 2.

The L and the G + L groups displayed higher CSD velocities of propagation ($P<0.05$), as compared to the C and G groups (in the case of L group), and as compared to the C, G and L groups (in the case of the G + L group). The means ± sd velocities of propagation (in mm/min) in the four groups were: group C, 3.45 ± 0.19; group G, 3.38 ± 0.20; group L, 3.78 ± 0.21; and group G + L, 4.37 ± 0.35. Data are illustrated in Fig. 3.

The fact that in the rat a single KCl stimulus elicits a single CSD episode is a usual observation. In contrast to that, in some occasions in the present experiments "multiple" CSD episodes appeared after a single KCl stimulation. These "multiple" CSDs are considered as evidence of a high brain susceptibility to the CSD phenomenon [30]. In the present study this was seen in 8 out of 14 C rats, 9 out of 13 G rats, 8 out of 13 L rats and 9 out of 12 G + L rats. Fig. 4 presents the number of "multiple" CSD expressed as medians and the 25%–75% intervals. The Kruskall–Wallis one way ANOVA on ranks indicated that the groups G and G + L displayed a significantly ($P<0.05$) higher number of multiple episodes of CSD (medians: 7 and 7.5 CSD-episodes, respectively) than the control and L groups, which presented comparable values (median equals to 3 in both groups).

3.3. Brain fatty acids measurements

Table 3 presents the levels of several fatty acids measured in brain homogenates of the 4 groups. One can see that the CLA molecules C18:2c9t11 and C18:2t10c12 were present in the three experimental groups, but not in the control rats.

4. Discussion

The main outcomes from this study were increased body weight and faster CSD propagation in the developing rats born from (group G), and/or suckled by dams fed the CLA-rich diet (groups L and G + L). In this experimental diet only the quality of the dietary lipid source was modified (fat from the goat milk, which is rich in CLA), as compared to the control diet (soybean oil). Regarding the effects of CLA on body weight, some reports from others have described weight loss in obese humans [31] and in suckling rats [32,33] treated with CLA. On the other hand, rats receiving a diet containing goat milk did not present changes in body weight [34], and pups from dams that received a CLA-supplemented diet during gestation and lactation displayed increases in body weight [35]. The discrepancy between our results and those from others may be due to methodological differences such as distinct CLA concentrations and sources (synthetic [31,32] versus goat milk in the present work), ages (adults [34] versus sucklings), and the health status of the organism (obese [31] versus eutrophic). The body weight loss in the CLA-treated groups has been attributed to a decrease of triglycerides in the lactating milk [32], or to a reduction in the synthesis of fatty acids by CLA action on lipogenic enzymes [33]. In our experiment, we used only the lipids from goat milk and CLA was present in this lipid source. The fat from goat milk contains several lipids including saturated, monounsaturated and polyunsaturated fatty acids. This possibly contributed to the increase in body weight of pups compared with the control group, which received a diet containing predominantly polyunsaturated fatty acids.

Because the measurement of physical parameters other than body weight required anesthesia, such measurement was performed only

Table 2
Physical parameters of 35–45 day old male rats, whose dams received the control diet containing soybean oil as the only fat source (control group) or the experimental diet containing fat from goat milk (which contains conjugated fatty acids), either during gestation, or lactation, or both gestation plus lactation periods. Data are expressed as mean ± sd. The number of animals in each group is 10. No intergroup significant differences could be detected.

Physical parameters	Groups			
	Control	Gestation	Lactation	Gest + Lact.
Body length (cm)	18.3 ± 0.9	17.4 ± 1.0	18.4 ± 0.6	18.4 ± 1.3
Abdominal circumference (AC; cm)	13.1 ± 1.3	12.4 ± 0.9	13.0 ± 0.5	13.5 ± 1.1
Thoracic circumference (TC; cm)	11.1 ± 1.2	10.8 ± 0.6	11.4 ± 0.5	11.7 ± 0.6
AC/TC ratio	1.18 ± 0.1	1.14 ± 0.1	1.16 ± 0.1	1.15 ± 0.1
Body mass index (g/cm^2)	0.49 ± 0.1	0.50 ± 0.1	0.47 ± 0.1	0.48 ± 0.1
Tail length (cm)	13.5 ± 1.2	13.2 ± 0.9	13.5 ± 1.4	14.3 ± 1.0
Brain weight (g)	1.63 ± 0.1	1.71 ± 0.1	1.67 ± 0.1	1.61 ± 0.1

at the end of the experiment (35–45 days of life). At that age, neither body weights, nor the other physical parameters in the CLA-treated animals presented any changes, as compared to the control group, suggesting that the CLA effects on body development were short-lasting. Notwithstanding the absence of weight difference, a significant CLA-associated enhancement in CSD propagation could still be demonstrated, suggesting a long lasting brain electrophysiological effect. This indicates a differential effect of CLA in the maternal diet regarding growth of the pups (short term effect) and CSD propagation (long term effect).

Regarding CSD, the increase in its speed of propagation was more evident when the maternal CLA-treatment occurred during lactation, as compared to the gestation period, and this effect was potentiated when the dams were treated during gestation plus lactation. Data raise the questions on why the lactation period is more vulnerable than the gestation period and how the CLA would be involved in

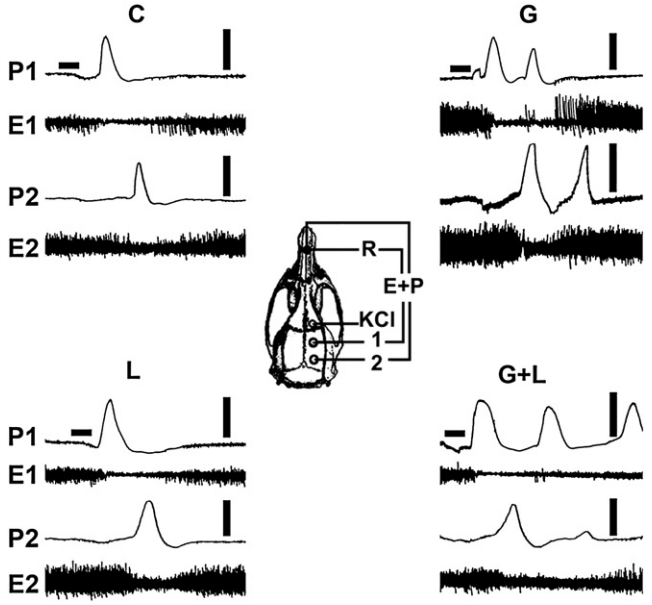

Fig. 2. Electrocorticogram (E) and slow potential change (P) recorded during cortical spreading depression (CSD), in four 35–45 day-old rats from the group control (C), and from the groups whose dams received the CLA-diet during gestation (G), or lactation (L), or both gestation + lactation (G + L). The horizontal bars in P1 show the period (1 min) of stimulation with 2% KCl, necessary to elicit CSD. The vertical bars equal −10 and −1 mV, respectively for the P and E recordings (negativity is upwards). The places of KCl application and of the reference electrode are indicated in the inset, which also shows the recording points 1 and 2 (from which the traces marked at the left with the same numbers were recorded). Note that in animals of the groups G and G + L a single KCl stimulation elicited two or more CSD episodes.

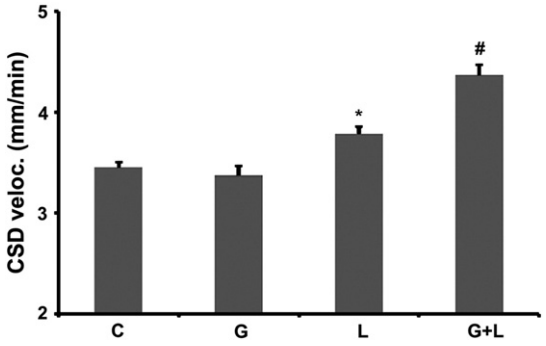

Fig. 3. Velocities of propagation of CSD in 35–45 day-old rats whose dams received the control diet (C), or the CLA-diet during the gestation (G), lactation (L) or both periods (G + L). The number of animals in the groups C, G, L and G + L is 14, 13, 13 and 12, respectively. Data are expressed as mean ± S.E.M. of twelve CSD episodes elicited, at 20-min intervals, by 1-min KCl application during the 4 h recording period. The group marked with one asterisk differs from the groups C and G. The group marked with (#) differs from all other three groups (ANOVA plus Tukey-test; $P<0.05$).

such developmental and electrophysiological effects. One possibility is that CLA would impair brain myelination, a developmental process that in mammals attains maximal intensity during the lactation [36]. Modifications in dietary lipids have been associated in the rat to changes in myelination mediators as mRNA, proteolipid protein and oligodendrocyte glycoprotein [37], and also to myelination-dependent auditory response conduction [38,39]. Interestingly, accelerated and decelerated CSD propagations have been associated with cortical demyelination and hypermyelination, respectively [40]. The facilitated CSD propagation shown in the present work stands in contrast with the CSD deceleration previously observed in rats treated during pregnancy and lactation with a diet rich in saturated fatty acids, which increases myelination [19]. In view of this evidence, we are tempted to suggest that the CLA-rich maternal diet used in this work probably impaired myelin synthesis in the progeny, and this would be causally involved in the CSD acceleration, as presently observed. In addition the increased incidence of "multiple" CSD episodes, after a single KCl stimulation, has been observed in the progenies from dams treated with CLA during the gestation period (groups G and G + L), rather than during lactation. This suggests that CLA acting on the two different developmental time points (gestation and lactation) affects distinct processes leading to the two CSD alterations, respectively increased incidence of multiple CSD, and higher CSD propagation velocities. Further specific experiments shall clarify this point.

Regarding the relationship between CSD and human neurological disorders, CSD has been postulated as representing an important pathophysiological hallmark for human migraine, and it has also been associated with epilepsy [27]. Interestingly, an increased prevalence of epilepsy is noted in patients with multiple sclerosis [41], a demyelinating disease [42], as compared to the general population. All the evidence notwithstanding, we believe that future experiments in which brain myelin will be quantified are required to definitely confirm our hypothesis.

Another possibility that could explain the effect of the CLA-rich diet in increasing CSD velocity would be based on the imbalance of the n−6:n−3 ratio in the brain of the CLA-treated rats. This possibility has been recently discussed by Borba et al. [19] in rats under dietary essential fatty acid (EFA) deficiency over two generations, based on the speculation that the n−6:n−3 imbalance could change cortical excitability by activating K^+ channels in neurons and by attenuating the oscillations in astrocytic Ca^{2+} concentration [43,44]. This excitability hypothesis raises the possibility that the finely balanced interactions between astrocytes and neurons could contribute for the facilitating effects induced by the CLA-rich diet upon CSD. In fact, it is known from the literature that disruption of the glia–neuron interaction induced by fluorocitrate can also facilitate CSD in the rat hippocampus

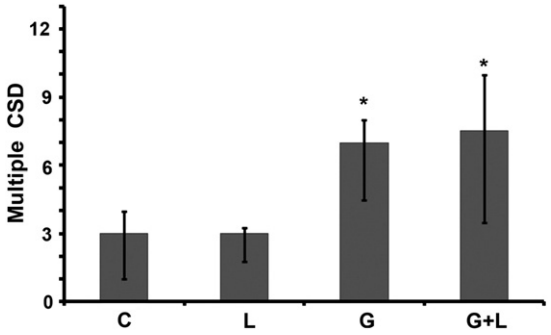

Fig. 4. Number of cases in which a single KCl stimulation for 1 min elicited multiple episodes of cortical spreading depression (CSD), in the four nutritional groups. C, G, L and G + L are groups composed from progenies of dams that received respectively the control diet (C), or the CLA-diet during the gestation period (G), or the lactation period (L), or both gestation + lactation (G + L). Multiple CSD episodes were presently observed in 8 out of 14 C rats, 9 out of 13 G rats, 8 out of 13 L rats and 9 out of 12 G + L rats. Data are expressed as medians, with the 25%–75% intervals. The groups marked with an asterisk are significantly different from the asterisk-free groups ($P<0.05$; Kruskall–Wallis followed by Dunn's test).

[45]. It is important to mention however, that compared with the control diet, our CLA-rich diet was deficient in both n − 3 and n − 6 fatty acids (proportionately more deficient in n − 6 than in n − 3 fatty acids), and this resulted in a 50% decrease of the n − 6:n − 3 ratio (from 22.52 in the control diet to 11.38 in the CLA-diet). The n − 6: n − 3 ratio is considered as an important nutritional factor for adequate brain development [2,6], and maternal diets containing different n − 6:n − 3 ratios have been associated with modifications in the time course of reflex ontogeny and in physical parameters in the rat progeny [4]. PUFA also may protect the brain from damage by inducing robust dilatation of the cerebral vessels [46]. More recently, Blondeau et al. [47] observed that linolenic- and docosahexanoic acid induced vasodilation of the basilar artery, and this could affect TREK-1 potassium channel. Because CSD is associated with increase in cerebral blood flow, besides modifications in extracellular levels of ions and neurotransmitters [48] one cannot discard the possibility that the linoleic acid content in the lipids from goat milk, used in the present study, in conjunction with other factors may facilitate the propagation of CSD as presently found.

Table 3
Brain fatty acids (as percent of total fatty acids) in 35–45 day old male rats, whose dams received the control diet containing soybean oil as the only fat source (group C), or the experimental diet containing fat from goat milk (which contains conjugated fatty acids), either during gestation, or lactation, or both gestation plus lactation periods (respectively groups G, L and G + L). Data are expressed as mean ± sd, and the number of animals in each group is 10. Two conjugated fatty acids, C18:2cis9-trans11 and C18:2trans10-cis12, are present in the brains of the experimental group, but not in the control rats. Asterisks indicate values that are significantly different from the corresponding control values.

Brain fatty acids	Groups			
	C	G	L	G + L
C16:1	28.37 ± 2.32	34.81 ± 4.05	27.89 ± 5.18	33.12 ± 7.1
C17:0	0.32 ± 0.04	0.40 ± 0.13	0.35 ± 0.09	0.35 ± 0.13
C18:0	0.29 ± 0.03	0.37 ± 0.07	0.39 ± 0.02	0.41 ± 0.03
C18:1n9t	25.04 ± 3.25	25.69 ± 3.12	23.81 ± 1.74	25.19 ± 3.79
C18:1n9c	1.19 ± 0.91	1.18 ± 0.65	1.35 ± 0.47	2.14 ± 2.69
C18:2n6c	19.02 ± 2.65	18.16 ± 3.87	18.57 ± 0.74	16.37 ± 1.15
C18:3n6	0.75 ± 0.08	0.91 ± 0.12	0.65 ± 0.15	0.68 ± 0.11
C18:2c9t11-CLA	0.00	0.75 ± 0.08*	1.38 ± 0.42*	1.85 ± 0.85*
C18:2t10c12-CLA	0.00	0.53 ± 0.06	0.60 ± 0.20*	1.25 ± 0.08*
C18:3n3	1.28 ± 0.42	0.96 ± 0.23	1.32 ± 0.29	1.06 ± 0.47
C20:4	8.58 ± 0.88	6.92 ± 1.89	8.01 ± 1.70	7.28 ± 1.66
C22:4	2.75 ± 0.37	1.54 ± 0.66	2.57 ± 0.78	1.98 ± 0.86
C22:6	7.87 ± 2.55	4.63 ± 2.87	8.93 ± 3.07	5.45 ± 1.59
ND	4.26	2.96	3.81	2.60

The idea that fatty acids would protect the neurons against glutamate-induced excitotoxicity is supported by evidence from in vivo experiments. Suckling rats whose dams were treated with dietary supplementation of fish oil, which is rich in Long Chain-PUFAs, displayed a reduction of NMDA-induced excitotoxic degeneration of cholinergic neurons [49]. Recently, a protective effect of CLA against glutamate excitotoxic action has been demonstrated in neuron cultures from mouse brain [17]. Considering that CSD is influenced by brain excitability changes [50,51], it is tempting to hypothesize that at least some of the CLA effects on CSD would involve CLA-dependent excitability alterations. Additional specific studies are needed to confirm this hypothesis.

In conclusion, our data documented, for the first time, a differential, facilitating effect of the lipids from goat milk on (1) body growth (a short-lasting effect), and on (2) an excitability-related phenomenon in the brain (CSD; long-lasting effect) that has causally been associated to important neurological disturbances like migraine and epilepsy. The data advance the understanding of the mechanisms by which dietary lipids can influence brain development and electrophysiological properties. Therefore, results may shed light on the comprehension of lipid-associated processes that may be involved in excitability-related neurological diseases.

Acknowledgements

The authors thank the financial support from the following Brazilian agencies: Empresa Brasileira de Pesquisas Agropecuárias (Embrapa), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Procad no. 131/2007), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministério da Saúde (SCTIE/DECIT no. 17/2006), Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (Facepe—APQ no. 0975-4.05/08), Instituto Brasileiro de Neurociências (IBN-Net/Finep no. 4191) and Instituto Nacional de Neurociência Translacional (INCT no. 573604/2008-8). R.C.A. Guedes is a Research Fellow from CNPq (no. 301190/2010-0).

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