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Research Article

Effect of maternal under-nutrition on pup body weight and hypothalamic endocannabinoid levels

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Abstract. Dietary long-chain polyunsaturated fatty acids are known to influence brain levels of the endocannabinoid anandamide in newborn pigs and mice. Furthermore, endocannabinoids were shown to control pup suckling and body weight in mice, and food intake in adult rodents. Here we determined the effect of maternal under-nutrition during gestation, lactation, or both, on body weight, and on the levels of endocannabinoids and expression of cannabinoid CB_1 receptors and fatty acid amide hydrolase in the hypothalamus of rat pups at weaning (21 days old) or adult rats (4 months old). Maternal under-nutrition resulted in a striking decrease in body weight of weaning rats, paralleled by a decrease in the hypothalamic levels of the endocannabinoid anandamide, but not of 2-arachidonoylglycerol. No significant change in the hypothalamic expression of either cannabinoid CB_1 receptors or fatty acid amide hydrolase mRNA was detected in any of the three groups of weaned pups. The decrease in pup body weight and hypothalamic anandamide levels was not observable in 4-month-old rats from any of the three groups. These data suggest that maternal under-nutrition causes a decrease in hypothalamic anandamide levels and loss of body weight, and confirm a crucial role for endocannabinoid signalling in neonatal development.

Key words. LCPUFA; anandamide; rat pup; perinatal under-nutrition; cannabinoid; food-intake.

All fatty acids have important functions, but the term essential is applied only to those long-chain polyunsaturated fatty acids (LCPUFAs) that are necessary for good health and cannot be completely synthesized in the body [1]. In fact, mammals cannot synthesize fatty acids with double bonds three (n-3) or six (n-6) carbons from the N terminus, such as arachidonic acid (20 : 4n-6, AA) and docosahexaenoic acid (22 : 6n-3, DHA), which are necessary for optimal visual acuity and brain development [2]. These LCPUFAs must be obtained from the diet. The need for AA, which is utilized for eicosanoid synthesis and is a constituent of membrane phospholipids involved in signal transduction, is one of the accepted reasons why the n-6 LCPUFAs are essential.

Eicosanoids include the endocannabinoids, anandamide (N-arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG) [3–5], which are endogenous ligands for the receptors of marijuana's active principle Δ^9-tetrahydrocannabinol [6], the cannabinoid receptors. To date, two cannabinoid receptors have been characterized: the type 1, or CB_1 [7], which is most concentrated in the central nervous system, but is present also in peripheral tissues, and the type 2, or CB_2, which seems to be restricted to the immune system [8]. The endocannabinoid system also comprises proteins for endocannabinoid biosynthesis and

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inactivation, such as the fatty acid amide hydrolase (FAAH) [9] and the anandamide transporter [10]. More recently, a third endocannabinoid, noladin ether, has been discovered [11].

Among the several possible physiological roles suggested so far for the endocannabinoid system, its likely participation in the orexigenic hypothalamic networks regulated by the anorexic hormone leptin appears particularly important [12]. In particular, hypothalamic endocannabinoid levels are decreased after systemic leptin administration in rats, and are increased in rodent models of hyperphagia and obesity, such as ob/ob and db/db mice, and Zucker rats, where leptin signalling is defective [12]. Furthermore, hypothalamic endocannabinoid levels increase in rats deprived of food [13]. These data, together with the finding of stimulation by endocannabinoids of food intake in both mice and rats [see ref. 14 for a review], and the discovery of an important role for CB_1 receptors in controlling food intake after prolonged food deprivation [12], strongly suggest that the endocannabinoid system plays a crucial role in the hypothalamic neural networks controlling appetite and body weight. This appears to be all the more true for rodents immediately after birth, since blockade of CB_1 receptors at post-natal day 1 results in total suppression of suckling and subsequent death of newborn mice [15].

LCPUFAs are also essential in the perinatal period, because the fetus and newborn mammals do not seem to synthesize sufficient amounts of AA and DHA from their precursors to cover their high needs [16]. In fact, the infant brain preferentially accretes LCPUFAs, and especially DHA and AA, during the last intrauterine trimester and the first month of life [17]. All the n-3 and n-6 fatty acids acquired by the fetus cross the placenta by simple diffusion and via the action of membrane-bound and cytosolic fatty-acid-binding proteins. Fetal blood is thus enriched in LCPUFAs in a way corresponding to the maternal supply [18]. The placenta may also regulate its own fatty acid substrate supply via the action of placental leptin on maternal adipose tissue. After birth, human milk contains LCPUFAs in sufficient amounts to meet infant requirements [19], but also 2-AG and traces of anandamide [20]. The mother's dietary status during gestation and lactation may affect the levels of these fatty acids and eicosanoids in maternal blood lipids and then in milk [21]. Furthermore, Berger and coworkers [22] have shown that brain anandamide and N-docosahexaenoylethanolamine levels are increased about fourfold when newborn piglets are fed a liquid formula supplemented with 20:4n-6 and 22:6n-3 fatty acids, in levels similar to those found in porcine milk, in comparison to piglets fed with the same liquid formula without supplementation of these fatty acids.

In the present study, we investigated the effect of maternal under-nutrition during late gestation (Pre), lactation (Post) or both periods (PP) on the levels of anandamide and 2-AG in the hypothalamus of both weaned and adult male rats in relation to possible changes in their body weight. Since a modulation of anandamide levels can also impact the expression of its brain receptor (CB_1) [23], and might be due to changes in the expression of FAAH [12], we compared the hypothalamic expression of both CB_1 and FAAH in weaned and adult male rats exposed to perinatal maternal undernutrition.

Materials and methods

Materials

Deuterated anandamide and 2-AG were synthesized from $[^2H]_8$-arachidonic acid and ethanolamine or glycerol as described previously [24].

Animals

Wistar rats (200 g) were purchased from IFFA-CREDO (L'Arbresle, France) and housed five per cage in a room with a controlled light cycle (L:D 12:12 h, lights on at 0700 hours) and temperature (22 ± 2 °C) with free access to food (regular rat chow, no. 113, containing 22% protein, 5% fat, 53% carbohydrate; UAR, Villemoisson sur Orge, France) and tap water ad libitum. After 8 days of acclimation, females were mated with a male for one night. The next day was taken as day 0 of pregnancy if spermatozoa were found in the vaginal smears. Pregnant females were then transferred into individual cages.

Animal use accreditation by the French Ministry of Agriculture (no. 04860) has been granted to our laboratory for experimentation with rats.

Feeding regimens

Four groups of pregnant rats were studied. A group of control (C) dams was fed ad libitum during gestation and the 3 weeks of lactation. Three groups of food-restricted females received 50% (FR50) of ad libitum intake, determined by the amount of food consumed by control females during gestation and lactation in a pilot study. The females of the first food-restricted group (Pre) received 12 g/day of food during the last week of gestation and were then allowed to eat ad libitum during lactation. The females of the second group (Post) were fed ad libitum until delivery and exposed to FR50 during lactation. The females of the third group (PP) were exposed to food restriction during the last week of gestation and until the end of lactation (21 days after delivery). For the dams of the Post and PP groups, the food supply was gradually increased when passing from the first to the third week of lactation. Food intake of dams gradually increases during lactation, and therefore the FR50 regimen had to take into account this physiological increase, so that FR50 dams always ate half of what control dams ate. In a pilot study,

this increase was precisely quantified during the 3 weeks of lactation. During the first week of lactation, the control regimen of the dams went from 24 to 44 g, food intake being gradually increased with an average increment of 3.4 g/day. Thus, during the same period, the regimen of FR50 dams ranged from 12 to 22 g with an average increase of 1.7 g/day. During the second week of lactation, control dams were fed from 54 to 62 g (average increment of 1.4 g/day) and FR50 dams from 27 to 31 g (0.7 g/day). Finally, during the last week of lactation, the regimens were as follows: control dams, from 70 to 80 g (1.8 g/day) and FR50 dams from 35 to 40 g (0.9 g/day). Water was always available ad libitum for all experimental groups.

At birth, litters were adjusted to eight pups per dam in both control and FR50 females. A small reduction of pup body weight at birth following a maternal FR50-type diet has been reported previously [40], and therefore neonatal body weight was not assessed. For studies at the age of 4 months, animals were housed four per cage until the day of sacrifice. Because of the animal size and the protocol of tissue collection, not all the physiological parameters can be measured for each pup. Therefore, only a limited number of newborns (n = 1–3) were used from each litter, according to the investigated parameters, to avoid litter effects.

Sacrifice and tissue collections

Male rat offspring were rapidly killed by decapitation between 09.00 and 10.00 hours at weaning (21 days after birth) and at the age of 4 months. The whole hypothalamus was dissected for endocannabinoids and mRNA extraction and stored at –70 °C until analysis.

Purification and quantification of endocannabinoids

The extraction, purification and quantification of anandamide and 2-AG from rat hypothalamus require a set of different biochemical steps [24]. First, tissues were dounce-homogenized and extracted with chloroform/methanol/Tris-HCl 50 mM pH 7.5 (2 : 1 : 1, v/v) containing internal standards (5 pmol of $[^2H]_8$ anandamide and 100 pmol of $[^2H]_8$-2-AG). The lipid-containing organic phase was dried down, weighed and pre-purified by open-bed chromatography on silica gel. The resultant fractions were obtained by eluting the column with 9 : 1 and 1 : 1 (by vol.) chloroform/methanol and then analyzed by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS) using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2010) quadrupole MS via a Shimadzu APCI interface.

MS analyses were carried out in the selected ion-monitoring (SIM) mode as described previously [23]. The temperature of the APCI source was 400 °C, the HPLC column was a Phenomenex (5 µm, 150 × 4.5 mm) reverse-phase column, eluted as described elsewhere [23]. Anandamide (retention time 16.0 min) and 2-AG quasi-molecular ions (m/z = 348.3, 379.3 and 300.3) were quantified by isotope dilution with the above-mentioned deuterated standards (same retention times and m/z = 356.3 and 387.3) and their amounts in picomoles or nanomoles normalized per milligram of lipid extract [23]. Two LC-MS peaks for both deuterated and undeuterated mono-arachidonoylglycerol were found at retention times of 17.0 and 18.9 min, respectively, corresponding to 2-AG and 1(3)-AG, in agreement with the previous observation that 2-AG undergoes isomerization during the purification procedure [25]. Therefore, the amounts of 2-AG were calculated by adding the amounts of the two isomers. Data were statistically evaluated by ANOVA (Bonferroni adjusted).

Total RNA isolation and semi-quantitative RT-PCR analysis

Total RNA from rat hypothalamus was extracted using Trizol reagent according to the manufacturer's recommendations (GibcoBRL). Following extraction, RNA was precipitated using ice-cold isopropanol, resuspended in water treated with diethyl pyrocarbonate (Sigma) and its integrity verified following separation by electrophoresis into a 1% agarose gel containing ethidium bromide. RNA was further treated with RNAse-free DNAse I (Ambion DNA-free kit) according to the manufacturer's recommendations to digest contaminating genomic DNA and to subsequently remove the DNAse and divalent cations.

The expression of mRNAs for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), FAAH and CB_1 receptors was examined by RT-PCR. Total RNA was reverse-transcribed using oligo dT primers. DNA amplifications were carried out in PCR buffer (Q-Biogen) containing 2 µl of cDNA, 500 µM dNTP, 2 mM $MgCl_2$, 0.8 µM of each primer and 0.5 U Taq polymerase (Q-Biogen). The thermal reaction profile consisted of a denaturation step at 94 °C for 1 min, annealing at 60 °C for 1 min and an extension step at 72 °C for 1 min. A final extension step of 10 min was carried out at 72 °C. The PCR cycles were 30 for CB_1, FAAH and GAPDH and were observed to be optimal and in the linear portion of the amplification curve (data not shown). Reactions were performed in a PE Gene Amp PCR System 9600 (Perkin Elmer). After reaction, the PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide for UV visualization.

The specific human oligonucleotides were synthesized on the basis of cloned rat cDNA sequences of GAPDH, FAAH and CB_1. For GAPDH, the primers sequences were 5′-CCCTTCATTGACCTCAACTACATGGT-3′ (nt 208–233, sense) and 5′-GAGGGCCATCCACAGTCTTCTG-3′ (nt 655–677, antisense). The FAAH sense and antisense primers were 5′-GTGGTGCT(G/A)ACCCCCATGCTGG-3′ (nt 469–475) and 5′-TCCACCTCC-

CGCATGAACCGCAGACA-3′ (nt 561–569), respectively. The CB_1 sense and antisense primers were 5′-GATGTCTTTGGGAAGATGAACAAGC-3′ (nt 365–373) and 5′-AGACGTGTCTGTGGACACAGACATGG-3′ (nt 460–468), respectively. The expected sizes of the amplicons were 470 bp for GAPDH, 300 bp for FAAH and 309 bp for CB_1. GAPDH housekeeping gene expression was used to evaluate any variation in the RNA content and cDNA synthesis in the different preparations. Furthermore, the PCR primers for GAPDH and FAAH were selected on the basis of the sequence of the FAAH gene (NCBI accession number AF098010) by including the introns 5476–6026 and 6173–6296, and of the sequence of the GAPDH gene (NCBI accession number AH007340) by including the introns 3216–3305, 3413–3541, 3633–3722, 3839–3930 and 4013–4205. In the presence of contaminant genomic DNA, the expected size of the amplicons would be 1062 bp for GAPDH and 1335 bp for FAAH. No PCR products were detected when the reverse transcriptase step was omitted (data not shown).

Statistical analyses

All data are presented as mean ± SE. Statistical analysis was performed using multiple analyses of variance (MANOVAs) followed by Dunnett's test or Bonferroni test. A p value of 0.05 or less was considered significant.

Results

Effect of maternal under-nutrition on pup weight

The effect of maternal under-nourishment during late gestation (Pre), lactation (Post) or both late gestation and lactation (PP) on pup body weight is shown in figure 1 A. A reduction in pup body weight was observed in weaned rats in all three restricted groups (Pre, Post, PP), although the effect was most dramatic in the Post and PP groups ($p < 0.01$). After 4 months, the pups from undernourished dams had recovered a body weight almost identical to the controls, although a reduction was still observed in the Post and PP groups (fig. 1 B).

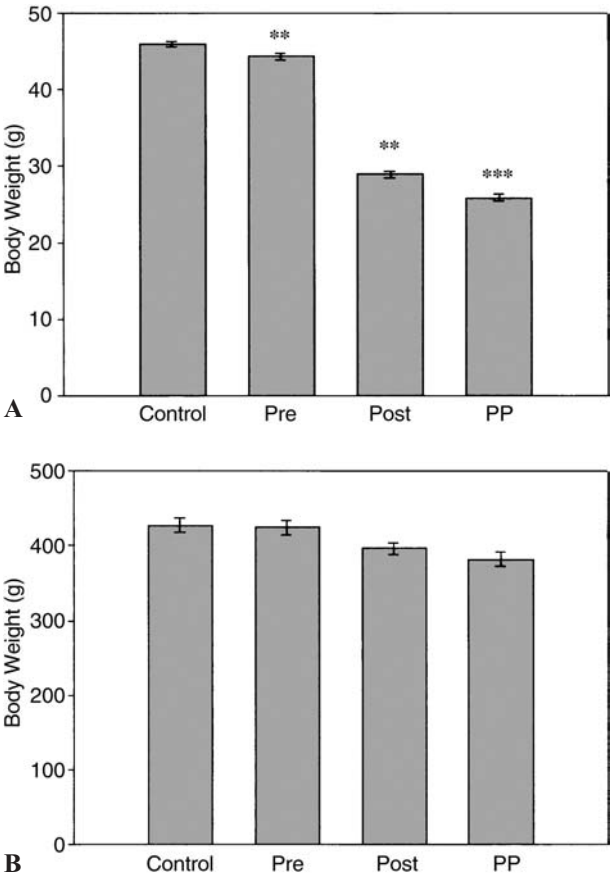

Figure 1. Modulation of body weight in pups from dams fed ad libitum (control group) or exposed to a 50% food restriction during late gestation (Pre group), during lactation (Post group) or during both gestation and lactation (PP group), at weaning (*A*) and at the age of 4 months (*B*). Data are expressed in grams and are means ± SE of n = 55. *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

Effect of maternal under-nutrition on endocannabinoid levels

To determine hypothalamic endocannabinoids levels in weaned rats in the control group and in the three groups of pups from the food-restricted dams (Pre, Post and PP), hypothalami were submitted to a lipid extraction in chloroform/methanol. A separation was then conducted using SiO_2 open-bed chromatography and the separated lipids (9:1 fraction) were finally subjected to LC-APCI-MS analysis. Control hypothalamic levels of anandamide were 0.70 ± 0.02 pmol/mg of lipid extract (mean ± SE, n = 3). 2-AG levels in the hypothalamus of newborn rats of the control group were 0.60 ± 0.017 nmol/mg of lipid extract (n = 3). Since the mother's dietary status can influence the levels of LCPUFAs and, subsequently, of the corresponding N-acylethanolamines in rat pup brain [22], we compared the amounts of these compounds in the hypothalamus of rats from the control diet group (that were considered as 100%) with those of the three groups of food-restricted dams (Pre, Post and PP) (fig. 2). Maternal under-nutrition during late gestation (Pre), during lactation (Post) and during both periods (PP) was accompanied by a significant decrease in anandamide levels in the hypothalamus of the corresponding rat pups to 78.57 ± 1.43%, 60.00 ± 2.86% and to 52.38 ± 9.05% of controls, respectively. Importantly, the effect in the pups from the Post and PP groups was significantly larger than in the Pre group ($p < 0.05$ in both cases, as assessed by ANOVA followed by the Bonferroni test). There were no significant changes in the levels of 2-AG.

Endocannabinoid levels were also measured in the hypothalamus of adult male rats (4 months old). In this case,

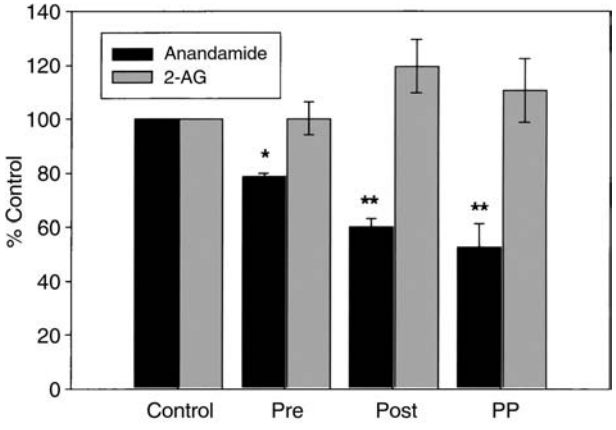

Figure 2. Modulation of the hypothalamic levels of anandamide and 2-AG in pups at weaning (21 days old) from dams fed ad libitum (control group) or exposed to 50% food restriction during late gestation (Pre group), during lactation (Post group) or during both late gestation and lactation (PP group). Data are expressed as percent of controls and are means ± SE of n = 4. Control levels were 0.70 ± 0.02 pmol/mg and 0.60 ± 0.017 nmol/mg of lipid extract for anandamide and 2-AG, respectively. *p < 0.05, **p < 0.01 vs control.

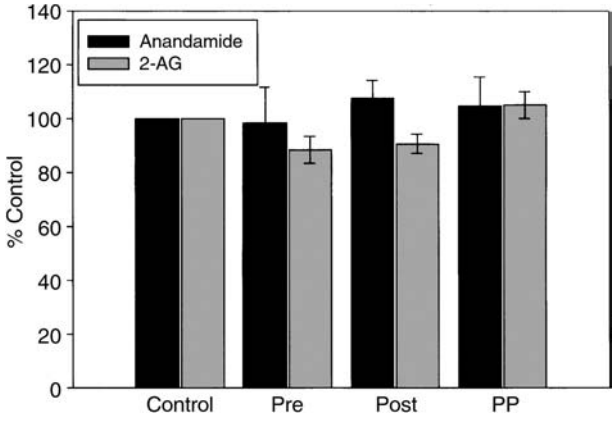

Figure 3. Modulation of the hypothalamic levels of anandamide and 2-arachidonylglycerol (2-AG) in adult rats (4 months old) from dams fed ad libitum (control group) or exposed to 50% food restriction during gestation (Pre group), during lactation (Post group) or during both gestation and lactation (PP group). Data are expressed as percent of controls and are means ± SE of n = 5. Control levels were 0.85 ± 0.09 pmol/mg and 0.27 ± 0.02 nmol/mg of lipid extract for anandamide and 2-AG.

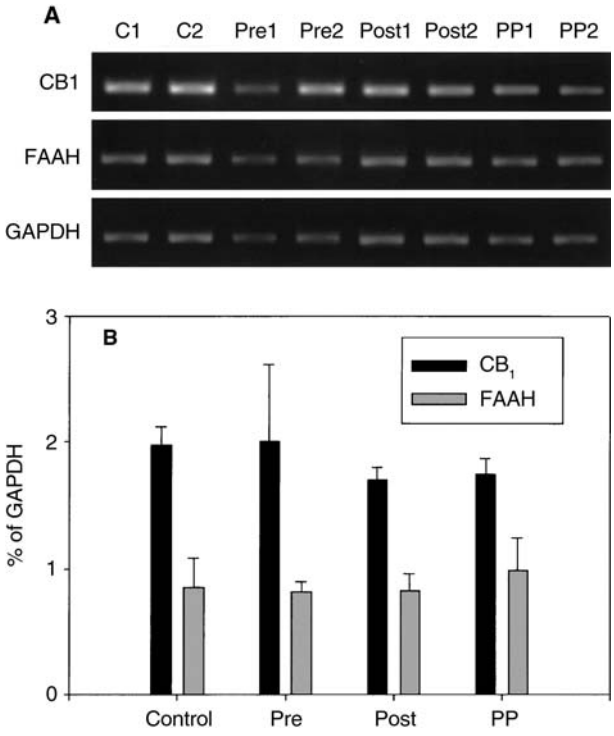

Figure 4. FAAH and CB_1 expression in the hypothalamus of weaned pups belonging to the control group (C) and to the three groups of food-restricted dams (Pre, Post and PP). (*A*) Intensity of the amplicon bands for FAAH and CB_1 mRNAs in pups of the control group (C1 and C2, lanes 1 and 2), or belonging to the food-restricted dams during late gestation (Pre, lanes 3 and 4), during lactation (Post, lanes 5 and 6) and during both periods (PP, lanes 7 and 8). GAPDH mRNA expression in hypothalamus of pups is shown as the housekeeping gene. The expected sizes of the amplicons were 300 bp for FAAH, 309 bp for CB_1 and 470 bp for GAPDH. (*B*) Densitometric analyses of the gels shown in *A*. Data were background subtracted and normalized to intensity of the GAPDH bands, and are means ± SD of n = 2 separate analyses.

no significant difference was observed between the control group and the three groups of rats from food-restricted dams (Pre, Post and PP) (fig. 3).

Effect of maternal under-nutrition on CB_1 and FAAH expression

We compared, using semi-quantitative RT-PCR, the expression of the mRNAs encoding CB_1 and FAAH in the hypothalamus of weaned rats of the four experimental groups. Using specific primers for rat CB_1 and FAAH, amplification of rat hypothalamus cDNA revealed the presence of mRNA transcripts of the expected length for CB_1 and FAAH in the hypothalamus of control pups. However, no significant difference between the control group and any of the three groups of food-restricted dams (Pre, Post and PP) was found in the mRNA transcripts for either CB_1 or FAAH (fig. 4).

Discussion

We have demonstrated for the first time that maternal under-nutrition during either late gestation, lactation or both is accompanied by a decrease in hypothalamic endocannabinoid levels in the pups at weaning. We observed that the levels of anandamide decrease in weaned pups from malnourished dams. Under-nutrition of the dams during lactation caused a larger decrease of this neuromodulatory substance than maternal under-nutrition imposed only during late gestation. When the dams were

malnourished during both periods, a further decrease in pup hypothalamic anandamide levels was observed. A strong reduction in pup body weight was also observed when the dams were under-nourished during lactation and during both late gestation and lactation. Indeed, there is a clear linear correlation between hypothalamic anandamide levels and body weight in the weaned rats of the four experimental groups. Therefore, in view of the inhibition of food intake and decrease in body weight upon blockade of cannabinoid CB_1 receptors previously observed in adult [12, 26] and, particularly, newborn [15] rodents, one might speculate that at least part of the reduction in body weight observed here in weaning rats is due to the reduction in their hypothalamic anandamide content and to the subsequent decrease in tonic CB_1 receptor stimulation. Accordingly, an almost entire recovery from both the loss of body weight and the decrease of hypothalamic anandamide levels was observed in adult rats at the age of 4 months.

We did not observe any change in the pup hypothalamic levels of the other endocannabinoid, 2-AG, following maternal undernourishment. This finding was not surprising since in a previous study, brain levels of this compound in lactating piglets and mice did not appear to be dependent on the presence of LCPUFAs in milk [22]. This is probably because 2-AG is produced from the hydrolysis of sn-2-arachidonate-containing phospholipids [12], whose brain levels in turn are not affected by the presence of higher amounts of AA or DHA in milk [22]. In adult rodents, of the two major endocannabinoids, 2-AG is the one whose brain levels seem to be more profoundly enhanced by food deprivation and leptin-impaired signalling [12, 13]. But the picture in newborn rodents is very likely to be different, with both anandamide and 2-AG having the same impact on food intake and body mass accumulation. In fact, administration of 2-AG (alone or together with substances that prevent its degradation) to post-natal mice is not sufficient to prevent the devastating effects on suckling and life expectancy caused by a single-dose injection at day 1 of the CB_1 antagonist SR141716A [15].

The assessment, in pups from under-nourished dams, of other parameters such as hormones, glucose and triacylglycerol plasma levels was not among the aims of the present study. However, the decrease in body weight observed here in weaning rats need not necessarily have been due to a decrease in food intake, but might also be ascribed to the down-regulation of other possible effects of hypothalamic anandamide, such as the release of pituitary hormones [see ref. 27 for a review], or the stimulation of several possible anabolic reactions [see ref. 28 for a review]. Indeed, a recent study in obese rats showed that blockade of endocannabinoid signalling causes only a transient effect on food intake and a more sustained and profound effect on adiposity [29]. The measurement of milk intake in rat pups, particularly from under-nourished dams, is not facilitated, as in mouse pups, by the presence of 'milk-bands' [15], and could have resulted in stress-related, non-specific effects. However, we did gain preliminary data indicating that maternal under-nutrition causes, in weaning pups, a decrease in fat depots and plasma levels of insulin, glucose and adrenocorticotropic hormone (ACTH) [30]. This latter finding might indeed be due to the decrease in hypothalamic anandamide observed in these animals, since intra-cerebral-ventricular injection of anandamide was previously found to increase plasma ACTH levels [31].

The reduction in anandamide levels does not seem to be accompanied by any change in the expression of CB_1 receptors, or of the anandamide-metabolizing enzyme, FAAH. The first of these findings suggests that, at least in the hypothalamus, CB_1 receptor expression is not under the direct control of anandamide levels, as previously postulated for the hippocampus and striatum, based on studies carried out using CB_1 receptor knockout mice [23]. The second finding, on the other hand, suggests that the decrease in the pup hypothalamic levels of anandamide following dam under-nutrition is not due to increased degradation of this compound. Instead, this decrease in anandamide content may result from a down-regulation of its biosynthesis in the hypothalamus, as previously suggested for the leptin-induced decrease of hypothalamic anandamide in adult rats [12].

The fatty acid composition of maternal blood and, subsequently, the type of diet of the mother, determines to a great extent the fatty acid composition delivered to the fetus [18]. Furthermore, the milk contains LCPUFAs, including small amounts of the ω-6 and ω-3 LCPUFAs, and its fatty acid composition is to a certain extent dependent on maternal diet [32, 33]. Also widely accepted is that the developing fetus depends mainly, if not completely, on the maternal supply of essential fatty acids [34]. Indeed, LCPUFAs are considered to be essential in the pre-natal and early post-natal periods, and because the synthesis of LCPUFAs from their precursors does not seem to cover the infant's high needs at this stage of development, the dietary supply of LCPUFAs from the mother becomes extremely important [35]. Berger and coworkers [22] have demonstrated that supplementation of control diets with AA (20 : 4n-6) and docosatetraenoic acid (22 : 4n-6) leads to a significant increase in anandamide and N-docosahexaenoylethanolamine levels in piglet brain. Conversely, piglets and mice fed diets lacking these fatty acids exhibited reduced brain amounts of anandamide [22]. By contrast, supplementation of diets with both 20 : 4n-6 and 22 : 4n-6 fatty acids did not lead to increased brain 2-AG levels [22]. Therefore, based on (i) the well-established dependence of pup LCPUFA supply on maternal fatty acid precursors and (ii) the previous observation that dietary LCPUFAs determine the brain lev-

els of anandamide, but not 2-AG, in newborn pigs and mice, a likely explanation for the reduced hypothalamic anandamide, but not 2-AG, levels in weaned rat pups, observed here, might be the decreased LCPUFA supply from both maternal blood (before birth) and milk (after birth). This decreased supply is expected to occur following under-nutrition of dams during late gestation and lactation, respectively, and is likely to result in a decrease in the ultimate biosynthetic precursors of anandamide, the sn-1-arachidonoyl-sn-2-acyl-phospholipids [36, 37], with a subsequent reduction in the output of the anandamide biosynthetic pathway. The finding of no changes in anandamide hypothalamic levels in adult rats at the age of 4 months is in agreement with this hypothesis. In fact, at the end of the suckling period, rats are not expected to be dependent on maternal fatty acid supply since they are normally on a self-sufficient diet and are able to satisfy their LCPUFA needs at this age. Accordingly, brain anandamide levels are known to increase progressively during the early postnatal period and to reach a maximum in the adult rat brain [38]. At least two experimental problems prevented us from conclusively demonstrating this hypothesis by analysing under-nourished dam milk for its LCPUFA content. First, the sampling of milk from under-nourished, lactating dams (which are very sensitive to handling) is extremely difficult. Second, relatively high amounts of milk would have to be sampled for an accurate estimation of its LCPUFA content. Therefore, until such experimental problems are solved, the observed decreased anandamide hypothalamic levels in pups from under-nourished dams might also be due also to factors other than a decreased LCPUFA content in the milk. Alternative, or possibly additional, explanations are supported by the observation that restriction of food intake of the dams during both gestation and lactation did not have additive effects on either anandamide levels or pup body weight. However, the lack of additivity of reduced Pre and Post maternal food intake could also be due to the possibility that only part of hypothalamic anandamide is influenced by the exogenous supply of LCPUFAs.

In conclusion, we have shown here that under-nourishment of rat dams during late pregnancy and lactation is accompanied by decreased anandamide levels in the hypothalamus of the pups at weaning, with a corresponding reduction in their body weight. Understanding whether there is a cause-effect relationship between maternal under-nutrition and pup hypothalamic endocannabinoid signalling and food intake, as well as the exact biochemical mechanism underlying the reduction in anandamide content, will require further studies. Furthermore, the recently suggested role of endocannabinoids in the regulation of feeding motivation and food palatability [39] also needs to be investigated in relation to neonatal food intake. At any rate, our data represent the first report of the impact of maternal under-nourishment on the neonatal endocannabinoid system, and are likely to open the way to several future studies on the role of hypothalamic endocannabinoids in infant development.

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