Undernutrition changes G_{D3} and G_{M2} synthase activities in developing rat hypothalamus

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G_{D3} (EC 2.4.99.8) and G_{M2} (2.4.1.92) synthases were determined in the hypothalamus of normal (diet: 25% casein) and pre- and postnatally undernourished rats (diet: 8% casein). Both enzymatic activities were statistically higher in the hypothalamus of undernourished rats than in the hypothalamus of control rats on the 21st gestational day. The results indicate that undernutrition similarly alters the activity of both of these ganglioside biosynthesis enzymes in the rat hypothalamus at the developmental ages studied. (J. Nutr. Biochem. 4:639–643, 1993.)

Keywords: protein malnutrition; ganglioside glycosyl-transferase; hypothalamus

Introduction

G_{D3} (EC 2.4.99.8) and G_{M2} (EC 2.4.1.92) synthases are the two key enzymes of ganglioside synthesis, and their activities change during the embryonic development of the rat brain.[1,2] Gangliosides, sialoglycosphingolipids involved in a variety of events in brain development,[3,4] are synthesized in a stepwise manner by transfer of carbohydrates from sugar nucleotide donors to glycolipid acceptors. This process occurs in the endoplasmic reticulum and Golgi apparatus.[5]

Several studies have shown that undernutrition alters central nervous system (CNS) gangliosides.[6–9] We recently demonstrated that early malnutrition reduces hypothalamic ganglioside concentration.[10] There are few reports on the effects of undernutrition imposed during gestation and/or lactation on the enzymes involved in CNS gangliosides metabolism, particularly in the hypothalamus.[7,11–13] The hypothalamus and hypophysis constitute a functional unit that integrates the central nervous and endocrine systems, and this functional unit regulates several physiological and behavioral activities that are modified by early undernutrition.[14,15]

The present report describes the changes in G_{D3} and G_{M2} synthase activities in the rat hypothalamus during development. It also shows, for the first time, the effects of undernutrition on these enzyme activities in the CNS.

Methods and materials

Chemicals

Triton CF54, Tween80, CMP-N-acetylneuraminic acid (CMP-NeuNAc), and UDP-N-acetylgalactosamine (UDP-GalNAc) were from Sigma Chemical Co. (St. Louis, MO USA). CMP-[4,5,6,7,8,9,^{14}C] sialic acid (CMP-[^{14}C]NeuNAc), sp. act. 9.47 Bq/mmol, was purchased from Amersham International (Berkinghamshire, UK). UDP-[6-^{3}H]-N-acetyl-D-galactosamine (UDP-[^{3}H]GalNAc), sp. act. 370 Bq/mmol, was from American Radiolabeled Chemicals (St. Louis, MO, USA). Ultima Gold was from Packard B.V. Chemical operators (Groningen, The Netherlands). All other chemicals and solvents used were of analytical grade.

Diets

The animals had free access to isocaloric diets (*Table 1*) containing 25% or 8% protein (casein), salts, and vitamins as

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Table 1 Percent (g/100 g diet) nutritional composition of the diets

Component	Casein diet	
	25%	8%
Casein (87% protein)	28.7	9.2
Fat (soybean oil)	15.0	15.0
Carbohydrate (corn starch)	50.15	69.65
Salt mix	4.0	4.0
Vitamin mix	1.0	1.0
Nonnutritive fiber	1.0	1.0
Energy (kcal/g diet)	4.3	4.3

These diets were supplemented with 0.15% L-methionine (Merck). Salt and vitamin compositions are according to Horwitz.[16]

recommended by the Association of Official Analytical Chemists[16] and as previously described by our group.[17,18]

Animals

Female Wistar rats from our breeding colony were fed diets containing 25% protein (control, normonourished group) or 8% protein (undernourished group) during pregnancy and lactation.[10] The litter size was adjusted to eight pups per mother on the first postpartum day.

Fetuses at 21 days of gestational age were obtained from females that were mated only once. The fetuses were delivered on day 21.5 of gestation (21.7 days to full gestation) by hysterectomy.[19] The birth process of pups may last 1–4 hours and several changes in energy sources and glycide and lipid metabolism have been detected in the pup brain during this short period of time.[19] Thus, to eliminate these variables, we opted for surgical delivery.

Enzyme preparation

Pools of eight hypothalami were homogenized in five volumes of 0.32 M sucrose/14 mM 2-mercaptoethanol (wt/vol). Homogenates were centrifuged at 1,000*g* (Sorvall centrifuge, RC-2B,SS-34 rotor, Du Pont Instruments, Newton, CT USA) for 10 min to remove debris. The resulting supernatant was centrifuged at 176,000*g* (Model L5-75B,SW 50.1 rotor, Beckman Instruments, Palo Alto, CA USA) for 1 hour. The resulting pellet, designated total particulate fraction, was resuspended in 0.32 M sucrose/14 mM 2-mercaptoethanol and used as the enzyme source.[20]

G_{M3} isolation

Gangliosides were obtained from dog spleen by extraction and partition according to the method of Folch,[21] and the upper phase was passed through a DEAE-Sephadex A-50 column (Pharmacia, Uppsala, Sweden). The monogangliosides were eluted with 5 column volumes of chloroform/methanol/0.08 M ammonium formate (30:60:8) (vol/vol). G_{M3} was further purified by thin layer chromatography (silica G-60; Merck, Darmstadt, Germany) using chloroform/methanol/0.25% $CaCl_2$ (60:35:8) (vol/vol) as developing solvent.[22] The G_{M3} band was scraped and eluted with chloroform/methanol/H_2O (5:5:1) (vol/vol),[23] and its NeuNAc content determined by the resorcinol method[24] as modified by Miettinen and Takki-Luukkainen.[25]

Enzyme activity determinations

G_{D3} synthase (EC 2.4.99.8) activity was determined in an incubation system containing 350 μM G_{M3} ganglioside, 200 μM CMP-[^{14}C]-NeuNAc (2 kBq), 50 μg Triton CF54, 24 mM $MnCl_2$, 150 mM sodium cacodylate/HCl buffer (pH 6.4), and 160 μg total particulate fraction protein as the enzyme source, in a final volume of 20 μL.

G_{M2} synthase (EC 2.4.1.92) activity was assayed in a similar incubation mixture containing 460 μM G_{M3} ganglioside, 64 μM UDP-[^{3}H]GalNAc (8.3 kBq), 72 μg Triton CF54/Tween 80 (2:1) (wt/wt), 24 mM $MnCl_2$, 150 mM sodium cacodylate/HCl buffer (pH 7.2), and 160 μg total particulate fraction protein as the enzyme source.

The systems were incubated for 150 min at 37° C. Under these conditions, product formation was proportional to incubation time and protein concentration. In both cases an assay was run in the absence of exogenous glycolipid acceptor to discount the incorporation into endogenous acceptors. Reactions were begun by the addition of the particulate fraction protein and were stopped by the addition of 0.5 mL 5% (wt/vol) trichloroacetic acid/0.5% phosphotungstic acid (TCA/PTA). The two enzymatic activities were determined as described by Maccioni et al.[1] Radioactivity was measured with an LKB 1209 Rackbeta liquid scintillation counter using Ultima Gold.

Protein determination

Protein contents were estimated by the method of Lowry et al.[26] Bovine serum albumin (Sigma Chemical) was used as the standard.

Statistical analysis

Data were analyzed statistically by two-way analysis of variance using an SPSS/PC-plus computer program. The Duncan multiple range test was used to compare means within diets. Student's *t* test was utilized to compare means between diets.

Results

G_{D3} synthase activity

G_{D3} synthase activity (*Figure 1*) measured in the hypothalamus of control and undernourished rats was significantly decreased from the 21st day of gestation to the 7th postnatal day.

By comparing the two diets, it was noted that G_{D3} synthase activity was statistically higher in the hypothalamus of undernourished animals than in the hypothalamus of control rats on the 21st gestational day.

G_{M2} synthase activity

Levels of G_{M2} synthase activity (*Figure 2*) determined in the hypothalamus of control rats on the 21st gestational day and 7th postnatal day were statistically similar and were significantly decreased from the 7th to the 14th postnatal day. In the hypothalamus of undernourished animals, G_{M2} synthase activity declined gradually during the period investigated.

By comparing the two diets, it was observed that G_{M2} synthase activity was statistically higher in the hypothalamus of undernourished rats than in the hypothalamus of control rats on the 21st gestational day.

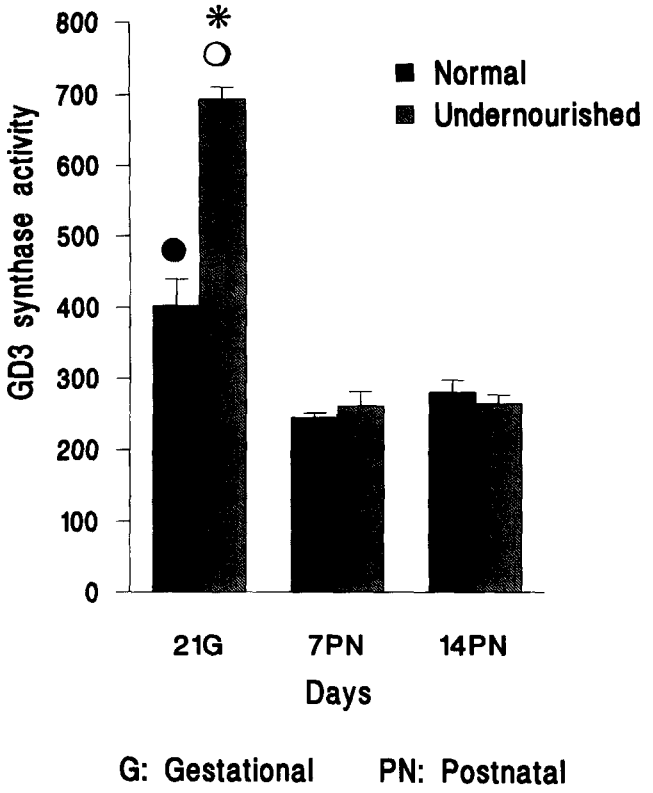

Figure 1 G_{D3} synthase activity in the hypothalamus of normal and undernourished rats. Bars represent mean ± SE of 3–9 determinations. Results are expressed as pmoles NeuNAc transferred/mg prot/150 min. (●) 21st gestational day value different from 7th and 14th postnatal days in normonourished rats ($P < 0.01$); (○) 21st gestational day value different from 7th and 14th postnatal days in undernourished rats ($P < 0.01$); (*) undernourished value different from normal ($P < 0.01$).

Discussion

The undernutrition model used in the present study caused a decrease in pup birth weight.[10] However, data from our department revealed that during the first two weeks of lactation nurse dams fed a diet containing 8% casein consume an amount of food that is statistically identical to that consumed by dams fed a diet containing 25% casein.[27] These data agree with those reported by Kanarek et al.[28] for gestational and lactational periods. These authors also observed that animals fed low protein diets (6% or 8%) were less efficient in transforming energy for weight gain than rats given a normal protein diet (25%).[28]

The last gestational week and the first three weeks of postnatal life represent the period of brain growth spurt.[29] This phase is characterized by intensive growth, differentiation, and branching of dendrites and axons, synaptogenesis, and myelinization.[29] When malnutrition is imposed during this period the process is markedly affected.[29]

Morgan and Naismith[11] observed that undernutrition imposed during the growth spurt reduced the peak activities of four enzymes that are known to participate in brain glycolipid metabolism, i.e., UDP-galactosyltransferase, UDP-glucosyl-transferase, CMP-N-acetylneuraminic acid synthetase, and sialidase. The attainment of peak activity was retarded by several days in the nutritionally deprived rats.

Gangliosides are sialoglycolipids involved in various events of brain development such as differentiation, neuritogenesis, synaptogenesis, and myelinization.[3,4] Several studies have shown that undernutrition alters central nervous system gangliosides.[6–13]

Developmental changes in ganglioside glycosyltransferases (G_{D3} and G_{M2} synthases) have been demonstrated in embryonic rat brain.[1,2] Major G_{D3} synthase activity has been observed during early embryonic stages, while an increase in G_{M2} synthase activity has been shown to occur during later embryogenesis and adulthood.[1,2] Similar results have been observed in embryonic chick retina[30] and embryonic human brain.[31]

The present study showed that G_{D3} and G_{M2} synthase activities were statistically higher in the hypothalamus of undernourished rats than in the hypothalamus of control rats on the 21st gestational day, indicating that undernutrition similarly alters both ganglioside biosynthesis reactions at the ages studied. Some investigators have shown that a low-protein diet results in decreased amino acid transfer from maternal to fetal blood in rats.[29] Pregnant rats and nursing rats (up to 2 weeks

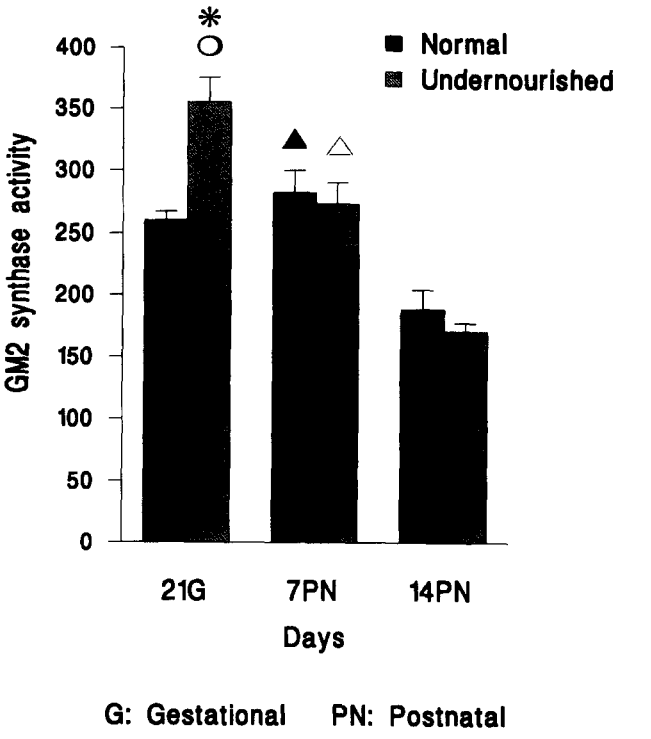

Figure 2 G_{M2} synthase activity in the hypothalamus of normal and undernourished rats. Bars represent mean ± SE of 3–6 determinations. Results are expressed as pmoles GalNAc transferred/mg prot/150 min. (○) 21st gestational day value different from 7th and 14th postnatal days in undernourished rats ($P < 0.01$), (▲) 7th postnatal day value different from 14th postnatal day in normonourished rats ($P < 0.01$); (△) 7th postnatal value different from 14th postnatal day in undernourished rats ($P < 0.05$); (*) undernourished value different from normal ($P < 0.05$).

of lactation) consume statistically identical amounts of diets containing different casein proportion (25 and 8%).[27,28] Thus, the carbohydrate to protein ratio is higher in the low-protein diet. This fact, however, may decrease the amount of milk produced rather than change the composition of maternal milk.[29] In a previous study on rats, we demonstrated that malnutrition reduced glycemia and increased hepatic glycogen concentration starting at 10 days of age.[32] These data agree with the glycide homeostasis detected by Kim et al.[33] and rule out the possibility of an effect of dietary carbohydrates on ganglioside synthesis.

In the present study, we showed that undernutrition causes changes in certain specific enzymes (G_{D3} and G_{M2} synthases). In a previous paper we reported that ganglioside content of the hypothalamus of pre- and postnatally protein-undernourished rats was lower than in control animals from the 7th day after birth.[10] These results, however, could be attributed to some modification in the contents of precursors of ganglioside biosynthesis in the undernourished rats, such as sphingosine, ceramide, glycosylceramide, lactosylceramide,[34-37] or donor sugar nucleotides (CMP-NeuNAc synthetase).[11]

The structural and biochemical changes occurring in the brain during the growth spurt can be retarded by undernutrition.[29] These phenomena may be so complex that they prevent the calculation of a precise correlation between quantitative changes in brain chemical constituents and the activities of enzymes that regulate their metabolism.[11]

Along this line of reasoning, we believe that present data may reflect a delay in hypothalamic development determined by undernutrition. This is also suggested by a comparison of the present results to the of G_{D3} and G_{M2} synthase activities detected in the brain from developing embryonic rats.[1,2]

If the present data could be extrapoled, even partially, to humans, they may perhaps be observed in the hypothalamus of small-for-gestational-age neonates[37] and in the hypothalamus of children with the clinical and biochemical characteristics of marasmus.[29,38]

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