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Undernutrition alters the hypothalamic ganglioside profile of the fetal rat

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In order to investigate the effect of protein undernutrition on the gangliosides profile, hypothalami of control and undernourished fetal rats were incubated with [6-^{3}H]glucosamine. Tissues were extracted with chloroform: methanol, and the purified ganglioside fractions were chromatographed on TLC plates. Chromatograms from both control and undernourished rats showed the presence of ganglioside bands that comigrated as G_{M1}, G_{D3}, G_{D1a}, G_{D1b}, G_{T1b}, and G_{Q1b}. When the two chromatograms were compared in terms of radioactive ganglioside distribution, the G_{D3} label was found to be higher in the hypothalamus of undernourished rats while the G_{D1a} label was higher in the hypothalamus of control rats. Since G_{D3} predominates during neuroblast and glioblast proliferation, while G_{D1a} is important during synaptogenesis, the present data may reflect a delay in hypothalamic development determined by protein undernutrition. (J. Nutr. Biochem. 6:155–157, 1995.)

Keywords: ganglioside; protein undernutrition; hypothalamus

Introduction

Several studies have correlated ganglioside concentration and composition with morphologic development in rodent, chicken, and human brains (recently reviewed by Rösner et al.[1]). Ganglioside of the lactosylceramide series, especially G_{D3}, are prevalent during the early developmental stages of the central nervous system (CNS) of birds and mammals; as development proceeds they are progressively replaced with gangliosides of the gangliotetraosylceramide series (G_{M1}, G_{D1a}, G_{D1b}, G_{T1b}).[2]

Besides the changes that occur with development, the content and profile of these glycosphingolipids are modified by neurometabolic disorders. It is well documented that various models of undernutrition alter CNS gangliosides.[3–7] In a previous paper we demonstrated that early malnutrition reduces hypothalamic ganglioside concentration between the 7th and 15th day of postnatal life[8] but does not affect their profile.*

However, we recently reported that the activities of the key enzymes for ganglioside biosynthesis (G_{D3} and G_{M2} synthases) are higher in the hypothalamus of undernourished rats that in the hypothalamus of controls on the 21st gestational day. This fact may represent a retardation in hypothalamic maturation determined by undernutrition.[9]

In view of these results, we undertook a study of the hypothalamic ganglioside profile in control and undernourished fetal rats on the 21st gestational day. Because it is very difficult to obtain enough material from these animals for this study we decided to use metabolic labeling with [^{3}H]glucosamine.

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Methods and materials

Chemicals

N-acetylneuraminic acid (NANA) and standard gangliosides were purchased from Sigma Chemical Co. (St. Louis, MO USA). TLC (silica gel 60) plates were from Merck (Darmstadt, Germany). D-[6-^{3}H]glucosamine (925 GBq/mmol) was from American Radiolabel Chemical Inc. (St. Louis, MO USA). Insta Gel was from Packard B.V. Chemical Operators (Gröningen, The Netherlands). All other chemicals and solvents used were of analytical grade.

Animals and diets

Female Wistar rats from our breeding colony were fed diets containing 25% protein (control, normonourished group) or 8% protein (undernourished group) during pregnancy (*Table 1*).[9] Fetuses at 21 days gestational age were obtained from females that were mated only one night. The fetuses were delivered on day 21.5 of gestation (21.7 days to full gestation) by hysterectomy. The birth process of pups may last 1–4 hr and several changes in energy sources and glucide and lipid metabolism have been detected in the pup brain during this short period of time. Thus, to eliminate these variables, we opted for surgical delivery.[10]

Biochemical procedures

Hipothalami from both groups of fetuses (2 control litters and 3 undernourished litters; ±300 mg) were incubated in 5 ml of KRB, 5 mM glucose, and 4.07 MBq of [^{3}H]glucosamine in a Dubnoff metabolic incubator at 37°C, in an atmosphere of O_2:CO_2 (95:5 v/v), for 150 min. The incubation medium was then separated from tissue by centrifugation at 1,000g for 10 min. Total lipids were extracted from the pellet according to the method of Roukema nad Heijlman,[11] and gangliosides were purified on a silicic acid column.[12] The chloroform:methanol:water (50:50:15) fraction was evaporated to dryness under N_2. The dry material was suspended in 2 ml of water and dialyzed three times for 4 hr each, against distilled water at 4°C. The dialysate was lyophilized and dissolved in 200 µl of chloroform:methanol (1:1).[13] Aliquots of 10 µl and 20 µl from this ganglioside fraction were utilized for radioactivity measurement with Insta Gel and for ganglioside quantification by the resorcinol-HC method, respectively.

Other samples of the ganglioside fraction (45,000 cpm/65 nmol NANA) were spotted on TLC plates and developed in chloroform: methanol:0.25% aqueous $CaCl_2$ (65:35:8 v/v).[12] The bands marked after exposure to iodine vapors were also visualized with the resorcinol-HCl reagent.[14,16] The areas of individual gangliosides were scraped off into vials and the silica was extracted with 0.5 ml of chloroform:methanol (1:1). Radioactivity was measured with and LKB 1209 Rackbeta liquid scintillation counter using Insta Gel.

Table 1 Percent (g/100 g of 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 of diet)	4.3	4.3

These diets were supplemented with 0.15% L-methionine (Merck). Salt and vitamin compositions are according to the official methods of analysis of the Association of Official Analytical Chemists.[9]

Results and discussion

[^{3}H]glucosamine incorporation into hypothalamic ganglioside fractions from control and undernourished fetuses was 624 cpm/nmol NANA and 758 cpm/nmol NANA, respectively.

As shown in *Figure 1*, the resorcinol-HCl method revealed 10 ganglioside bands. This number was higher than that cited in the literature for normal and undernourished fetal brains.[3,17,18] Six bands comigrated with the following standards: G_{M1}, G_{D3}, G_{D1a}, G_{D1b}, G_{T1b}, and G_{Q1b}. The ganglioside between G_{D1a} and G_{D1b} (band 5) may be a ganglioside characterized as G_{D1a}-Gal-NAc, or G_{T1a}, or G_{D2}.[18–20] Furthermore, two other bands were visualized between G_{D1b} and G_{T1b} (bands 7 and 8) one of them probably corresponding to ganglioside G_{T1L}.[20,21]

Table 2 shows the ganglioside distribution expressed as a percentage of labeling ganglioside separated by TLC (*Figure 1*). Tetrasialic gangliosides presented the highest radio-

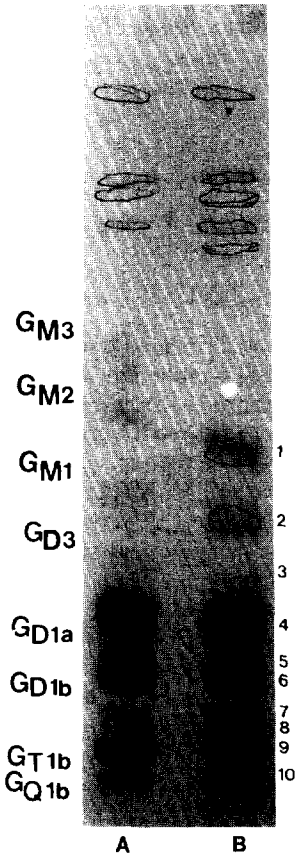

Figure 1 TLC of the labeled gangliosides from the hypothalamus of control (A) and undernourished (B) rats on the 21st gestational day. The mobilities of standard gangliosides submitted to the same TLC are indicated on the left. The algarisms on the right correspond to bands visualized with the resorcinol-HCl reagent. The bands marked in pencil indicate previous iodine exposure.

Table 2 Percent distribution of radioactivity detected in the ganglioside bands illustrated in *Figure 1*

Band	Control	Undernourished	Equivalent standard
1	2.9	2.5	G_{M1}
2	4.1	21.1	G_{D3}
3	4.3	3.6	
4	11.3	5.2	G_{D1a}
5	5.7	3.5	
6	6.4	6.2	G_{D1b}
7	4.5	5.5	
8	5.0	4.5	
9	15.4	11.7	G_{T1b}
10	40.5	36.2	G_{Q1b}

The total quantity of cpm detected in each of the two chromatograms in *Figure 1* was considered to be 100% radioactivity.

active incorporation. By comparing the radioactive distribution of the two chromatograms (control and undernourished), it was noted that the G_{D3} label was higher in the hypothalamus of undernourished fetal rats than in the hypothalamus of control fetal rats. In contrast the G_{D1a}[3] label was higher in the hypothalamus of control fetal rats than in the hypothalamus of undernourished fetal rats. Similar results were obtained using D-[^{3}H]N-acetyl-mannosamine as a radioactive precursor (data not shown).

Altaman and Bayer[22] demonstrated that neurons of the various hypothalamic nuclei originate between the 12th and 18th embryonic day. The hypothalamus can form the first synapses as early as during the last week of gestastion.[23] It is known that G_{D3} predominates during neuroblast and glioblast proliferation, while G_{D1a} is important during synaptogenesis.[1] Developmental changes in ganglioside composition have been reported in embryonic rat brain, with G_{D3} and G_{D1a} contents, respectively, decreasing and increasing after the 16th embryonic day.[17,18] Biochemical, structural, and functional changes occurring in brain embryogenesis, however, can be retarded by undernutrition.[24] On this basis we believe that the present observations, especially concerning G_{D3} and G_{D1a} contents, may reflect a delay in hypothalamic neuronal and/or glial genesis and later synaptogenesis determined by protein undernutrition.

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