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Effects of Undernutrition on Glycine Metabolism in the Cerebellum of Rats

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Key Words
Protein malnutrition · Glycine metabolism · Cerebellum

Abstract
Undernutrition is a worldwide problem affecting millions of unborn and young children during the most vulnerable stages of brain development. Total restriction of protein during the perinatal period of life can alter the development of the mammalian fetus and have marked repercussions on development of the central nervous system (CNS). The brain is vulnerable to undernutrition with altered morphologic and biochemical maturation, leading to impaired functions. The focus of this study is to investigate [U-^{14}C]glycine metabolism in undernourished rats submitted to pre- and postnatal protein deprivation (diet: 8% protein with and without addition of *L*-methionine; control group: 25% protein). Although undernutrition produced a reduction in cerebellar weight and alterations in the DNA concentration, the present study shows that glycine metabolism in this structure is partially protected because the undernourished group with *L*-methionine did not show modifications in glycine metabolism at all ages studied. However, *L*-methionine deficiency alters glycine metabolism at 7 and 21 days, but in the adult age both undernourished groups presented no differences in oxidation to CO_2, conversion to lipids and incorporation into protein from glycine, compared to the control group.

Introduction

There is some doubt that a quantitative deficit or a qualitative imbalance in food intake produces alterations in nervous system ontogeny and function [1]. Several ontogenic steps of brain development, including neuronal proliferation and migration, brain growth spurt and myelination, are altered by nutritional insults resulting in long-lasting or even permanent deleterious effects [2, 3].

In precocial animals (such as the chick and guinea pig), the cerebellum is well developed at birth, whereas in atricial animals (rodents and man), the cerebellum is immature and its histogenesis and morphogenesis mainly occur during the postnatal period. Only around 3% of the cerebellar cells found in the adult are present in the rat at birth [1].

Glycine is a metabolic precursor for the synthesis of various low molecular weight compounds like purine bases, creatine, serine, tripeptide glutathione (γ-glutamylcysteinylglycine) and δ-aminolevulinate. In the CNS glycine serves as an inhibitory neurotransmitter and modula-

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tor of NMDA receptors [3, 4]. Although glycine metabolism in the brain remains to be clarified, there are at least two enzymatic systems detected in astrocytes: the glycine cleavage system (GCS; EC 2.1.2.10) [5], and serine hydroxymethyltransferase (SHMT; EC 2.1.2.1) [6]. Astroglial cells can degrade glycine by the GCS, and it is apparently lacking in neurons [7, 8]. Dringen et al. [9] demonstrated that astroglial cells are able to synthesize creatine, serine and glutathione using glycine as precursor.

Methionine is required for the synthesis of S-adenosylmethionine. A lack of methionine can affect protein synthesis and S-adenosylmethionine formation which are essential for the synthesis of glutathione and compounds that contain methyl groups.

The amino acid glycine is more oxidized in the rat cerebellum [10], a structure susceptible to undernutrition [1]. This study evaluated the effects of undernutrition on glycine metabolism and DNA concentration in the cerebella of rats at different ages.

Materials and Methods

Chemicals

Chloroform, formic acid and methanol were obtained from Merck SA (Porto Alegre, Brazil), and [U-^{14}C]glycine was from Amersham International (Little Chalfont, UK). All other chemicals were of analytical grade.

Animals

Albino Wistar rats were obtained from the Departamento de Bioquímica/UFRGS, and were maintained at 22 °C/12-hour light-dark cycle. Female Wistar rats were fed diets containing 25 or 8% protein (with or without addition of *L*-methionine) during the entire pregnancy and lactation, whereas additional undernutrition for up to 75 days was induced by maintaining the same 8% diets. Litter size was limited to 8 pups/dam.

Diets

The diets were as follows: (1) 25% protein (control group), (2) 8% protein with *L*-methionine, and (3) 8% protein without *L*-methionine. The animals had free access to isocaloric diets, and salt and vitamins as recommended by the Association of Official Analytical Chemists [11] and previously described by our group [12].

Experimental Procedure

To measure oxidation to CO_2 and the incorporation of lipids and protein synthesis from [U-^{14}C]glycine, 7-, 21- and 75-day-old rats were used and killed by decapitation. The cerebella were isolated, weighed and cut into 0.3-mm slices using a McIlwain tissue chopper. Between 50 and 60 mg of tissue slices were incubated in: 1.0 ml Krebs Ringer bicarbonate buffer (pH 7.4) + 5 m*M* glucose + 0.2 m*M* glycine + 0.5 μCi [U-^{14}C]glycine. Incubations were carried out in flasks after the contents had been gassed with a 95% O_2:5% CO_2 mixture for 30 s and then sealed with rubber caps. The slices were incubated at 35 °C for 1 h in a Dubnoff metabolic shaker (60 cycles/min) according to Dunlop et al. [13]. Incubation was stopped by adding 0.2 ml 50% TCA through the rubber caps. Then 0.2 ml of 2 *M* NaOH was injected into the central wells. The flasks were shaken for further 30 min at 35 °C to trap CO_2. Thereafter, the contents of the central wells were transferred to vials and assayed for CO_2 radioactivity in a liquid-scintillation counter. The flask contents were homogenized, transferred to tubes and washed three times with 10% TCA. The lipids were extracted with 2:1 chloroform:methanol (C:M). The C:M phase was evaporated in vials and the radioactivity measured. The precipitate resulting after washing with 2:1 C:M was dissolved in concentrated formic acid and radioactivity measured. This radioactivity represents protein synthesis from [U-^{14}C]glycine. All the results are expressed considering the initial specific activity of the incubation medium. The CO_2 production rate as well as the incorporation of radioactivity into lipids and protein was constant through 30, 60 and 90 min of the incubation period. The DNA concentration was determined by the method of Burton [14]. The protocol of this research was set up according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil. Data were analyzed statistically by ANOVA and by the Duncan multiple-range test, and by Student's t test when indicated, with the level of significance set at $p < 0.05$.

Results

In this study we investigated the effects of undernutrition on oxidation to CO_2, incorporation to lipids and into protein synthesis from [U-^{14}C]glycine in the cerebella of 7-, 21- and 75-day-old rats. At 7 and 21 days the undernourished group without *L*-methionine had higher [U-^{14}C]glycine oxidation, conversion to lipids and protein synthesis than the control group (fig. 1–3). However, the undernourished group with *L*-methionine presented no difference from the control group at all ages studied. In the adult age (75 days) there was no difference between the 3 groups on these parameters.

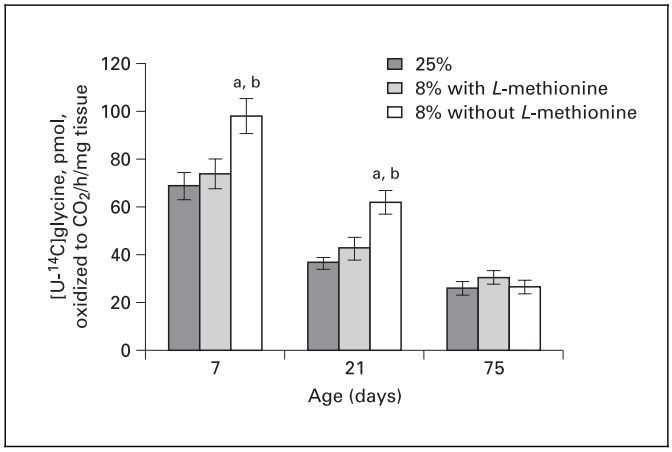
Table 1. Effects of undernutrition on cerebellar weight (g) of rats

Age days	25% protein	8% protein with *L*-methionine	8% protein without *L*-methionine
7	64.57 ± 1.65	55.88 ± 0.74[a, b]	48.50 ± 0.53[a]
21	202.88 ± 2.63	188.25 ± 2.62[a, b]	171.00 ± 4.05[a]
75	285.14 ± 3.33	232.89 ± 4.21[a, b]	181.80 ± 3.10[a]

Values are expressed as mean ± SEM. Each group comprised 18 animals.

[a] Statistically different from the control group ($p < 0.05$).

[b] Statistically different from the 8% protein group without addition of *L*-methionine ($p < 0.05$).



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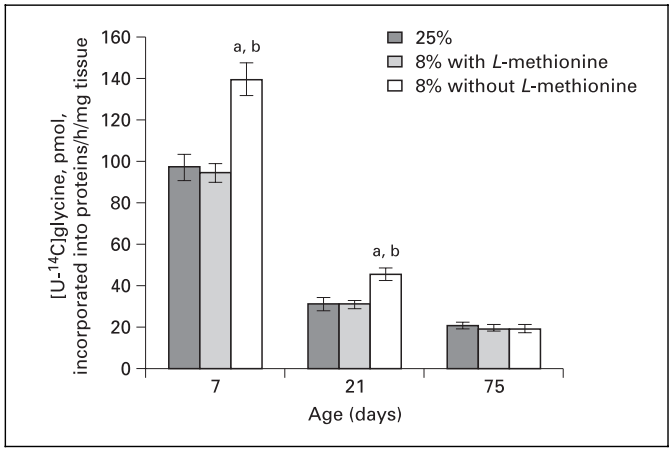

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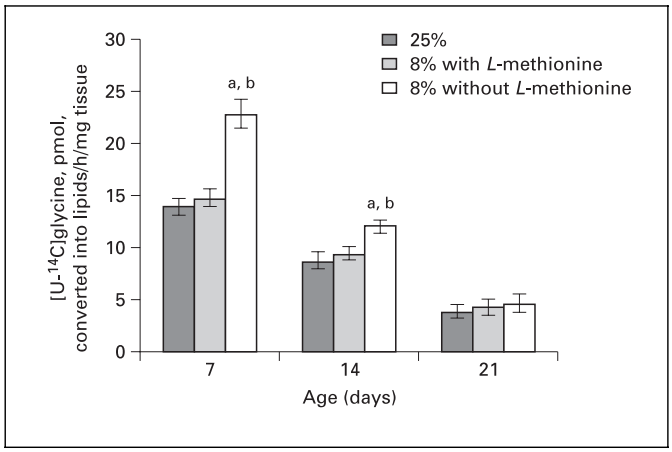

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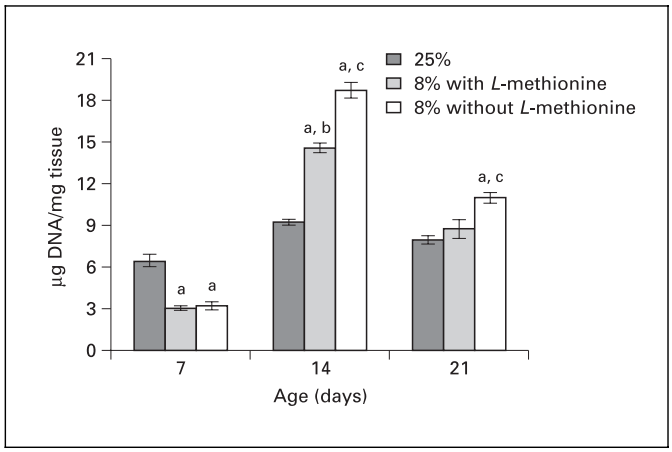

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Fig. 1. Oxidation of [U-^{14}C]glycine in cerebellar slices from 7-, 21- and 75-day-old rats. Cerebella were incubated with 0.2 m*M* of glycine and 0.5 µCi [U-^{14}C]glycine according to the Materials and Methods. Values are expressed as mean ± SEM. The number in each group is 12. [a] Statistically different from the control group ($p < 0.05$); [b] statistically different from the group fed the diet with 8% protein and *L*-methionine ($p < 0.05$).

Fig. 2. Conversion of [U-^{14}C]glycine to lipids in cerebella from 7-, 21- and 75-day-old rats. Cerebellar slices were incubated with 0.2 m*M* glycine and 0.5 µCi [U-^{14}C]glycine according to the Materials and Methods. Values are expressed as mean ± SEM. The number in each group is 12. [a] Statistically different from the control group ($p < 0.05$); [b] statistically different from the group fed the diet with 8% protein and *L*-methionine ($p < 0.05$).

Fig. 3. Incorporation of [U-^{14}C]glycine into proteins in cerebella from 7-, 21- and 75-day-old rats. Cerebellar slices were incubated with 0.2 m*M* of glycine and 0.5 µCi [U-^{14}C]glycine according to the Materials and Methods. Values are expressed as mean ± SEM. The number in each group is 12. [a] Statistically different from the control group ($p < 0.05$); [b] statistically different from the group fed the diet with 8% protein and *L*-methionine ($p < 0.05$).

Fig. 4. Concentration of DNA per milligrams of cerebellum of 7-, 21- and 75-day-old rats. Value are expressed as mean ± SEM. The number in each group is 18. [a] Statistically different from the control group ($p < 0.05$); [b] statistically different from the group fed the diet with 8% protein without addition of *L*-methionine ($p < 0.05$); [c] statistically different from the group fed the diet with 8% protein and *L*-methionine ($p < 0.05$).

All diet groups have different cerebellar weights and the degrees of undernutrition were inversely proportional to the cerebellar weights at all ages studied (table 1).

At 7 days both undernourished groups had a lower DNA concentration, whereas at 21 days the DNA concentration in the undernourished group without *L*-methionine was higher than in the control group, with the group without *L*-methionine showing the highest DNA concentration (fig. 4). At 75 days the DNA concentration in the undernourished group without *L*-methionine addition was higher than in both the other groups studied. At this age the malnourished group with *L*-methionine was not different from the control group.

Discussion

The main pathway for glycine oxidation in many CNS regions is the GCS. Previously [11] we measured CO_2 production from glycine using [1-^{14}C]glycine because the GCS releases the radioactive marker as CO_2, and showed that this cleavage is about 80 times greater than the oxidation of [2-^{14}C]glycine in the cerebella of 14-day-old rats. The contribution of glycine oxidation through serine (by SHMT) is practically insignificant in 14-day-old and adults rats (60 days) [5, 11].

To synthesize neutral lipids, glycine must be previously converted to serine (by SHMT), which can sequentially produce pyruvate, acetyl-CoA plus CO_2, and finally neutral lipids and CO_2. Fagundes et al. [10] showed that the lipids synthesized from glycine and serine in the CNS are predominantly phospholipids.

Glycine metabolism is higher at 7 days than at the other ages studied (fig. 1–3). Rotta et al. [15] found that with the increase in animal age there was a decrease in glycine oxidation to CO_2 by GCS, diminishing NH_3 formation in the CNS. This is important because glutamine catabolism in the CNS is higher at adult age. With the increase in animal age, glutamine oxidation increased while glycine oxidation decreased, contributing to the prevention of the elevation in ammonium concentration. The same authors showed that glycine was the poorest amino acid converted to lipid in cerebellar slices and this could be explained by the fact that acetyl-CoA synthesis from serine has no physiological significance. In the cerebella of 14-day-old rats, Fagundes et al. [10] observed a higher protein synthesis from [1-^{14}C]glycine and conversion to lipids than in adult rats because development of this brain region basically occurs postnatally, with the most rapid growth occurring between 0 and 15 days of postnatal life [16].

As observed by Gourdon et al. [17], undernutrition produces a reduction in cerebellar weight (table 1), and the weight was lower according to the severity of undernutrition, therefore the group without *L*-methionine presented the lowest cerebellar weights.

Azzolin et al. [18] found that the cerebellar DNA concentration was higher in normally fed 7- and 15-day-old rats than in undernourished rats, whereas at 21 days it was higher in undernourished rats, in agreement with this study to the age of 7 days (fig. 4). At 21 days, the undernourished groups had a higher DNA concentration, with a delay in the cellular division of cerebella from rats fed a low-protein diet. The undernourished group without *L*-methionine had a higher DNA concentration compared to the other groups at the age of 75 days. Similar data were shown by Gourdon et al. [17] using another experimental model of undernutrition, when the cerebellar DNA concentration of control rats at 4, 10 and 12 days of age was higher than that of the undernourished group. However, these authors could not find a difference between the groups at 21 days of age. Zamanhof et al. [19] found reduced cell division in all undernourished fetal organs studied, including the brain. Perinatal undernutrition acts by delaying cell division with prolongation of total cell cycle time. Studies by Shimada et al. [20] showed that prenatal undernutrition significantly extended the generation time of matrix cells in undernourished mice, resulting in a 14% prolongation of the generation time of these neuron precursor cells, thereby decreasing neuron production.

Although undernutrition produces structural alterations in the cerebellum, the present study shows that glycine metabolism in this structure is partially protected because the undernourished group with *L*-methionine did not show modifications in glycine metabolism at all ages studied. However, *L*-methionine deficiency alters glycine metabolism at 7 and 21 days. In the adult age, both undernourished groups did not present modifications in glycine metabolism showing that undernutrition could affect CNS development at different levels; however, plasticity phenomena or compensatory mechanisms could produce functional restoration [21].

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