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Ontogenetic Study of the Effects of Energetic Nutrients on Amino Acid Metabolism of Rat Cerebral Cortex

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We studied the effect of various energetic nutrients on metabolism of L-[U-^{14}C]leucine and [1-^{14}C]glycine in cerebral cortex of rats at different ages. At gestational age, glucose and lactate stimulated protein synthesis from L-[U-^{14}C]leucine and [1-^{14}C]glycine and from L-[U-^{14}C] leucine, respectively; glucose, β-OH-butyrate and lactate stimulated lipid synthesis from L-[U-^{14}C] leucine. At 10 days of age, glucose, mannose, and fructose stimulated protein synthesis, and glucose and mannose stimulated oxidation to CO_2 as well as lipid synthesis from L-[U-^{14}C]leucine. In adult rats, glucose, mannose, and fructose stimulated protein synthesis from L-[U-^{14}C]leucine and [1-^{14}C]glycine; glutamine also markedly decreased the oxidation of L-[U-^{14}C]leucine and [1-^{14}C]glycine in 10-day-old and adult rats.

KEY WORDS: Cerebral cortex; ontogenesis; amino acid metabolism; energetic nutrients.

INTRODUCTION

The brain is capable of utilizing a variety of substrates for energy production, including glucose (1–3), ketone bodies (2–6), glycerol, and glutamine (2,5,7). The utilization of nutrients by the central nervous system (CNS) can be regulated at several levels such as transport by capillary endothelial cells (8), transport through plasma membrane (9,10), variations in enzyme activity (11,12), and variations in plasma nutrient concentrations (13,14).

Additionally to the known role of these nutrients on brain energy metabolism as reported above, it has been demonstrated that they can modulate amino acid metabolism. Chaplin et al. (15) have shown, in brain slices from adult rats, that omission of glucose from the incubation or the addition of amino acids, at concentrations normally present in rat plasma, resulted in a significant reduction in L-[1-^{14}C]leucine oxidation and incorporation to protein. Patel and Owen (16) have shown that 5 mM glucose stimulated the incorporation of L-[U-^{14}C]-leucine to proteins and lipids and oxidation to CO_2 in cerebral cortex from 7-day-old rats; in adult rats, the same effects on lipids and protein metabolism were observed, but not on CO_2 production. Studying the interaction of leucine, glucose and ketone metabolism in rat brain in vitro, Palaiologos et al. (17), working with adult rats, have demonstrated the stimulatory effect of glucose on L-[U-^{14}C]leucine and L-[1-

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^{14}C]leucine oxidation to CO_2 and β-OH-butyrate did not present the stimulatory effect on L-[U-^{14}C]leucine oxidation to CO_2. Using [^{15}N]leucine as marker, Yudkoff et al. (18) showed that approximately one third of $-NH_2$ group of glutamine originates from leucine by transamination and possibly the addition of glutamine to the incubation medium decreases the formation of α-keto-isocaproate and further oxidation to CO_2.

The objective of the present work is to verify the effect of energetic nutrients on cerebral protein and lipid synthesis from leucine and glycine at different ages, as well as the oxidation of both amino acids to CO_2. We have chosen mannose and fructose because there were no references in the literature regarding the effect of these sugars on amino acid metabolism in the CNS. We used lactate, β-OH-butyrate, glycerol, and glutamine because their oxidation to CO_2 in the CNS varies markedly with the age of the animals.

EXPERIMENTAL PROCEDURE

Materials. Chloroform, formic acid, and methanol were obtained from Merck SA, Porto Alegre, Brazil. Hyamine hydroxide was purchased from J. T. Baker Chemical Company, Phillisburg, NJ, USA. L-[U-^{14}C]leucine and [1-^{14}C]glycine were from Amersham International (Berkinghamshire, U.K.), and [2-^{2}C]glycine [2-^{14}C] from Dupont NEN (Boston, MA).

Animals. Albino Wistar rats of different ages (21 gestational days and 10, 21, and 90–100 postnatal days) were obtained from the Instituto de Ciências Básicas da Saúde, UFRGS, and fed on stock laboratory diet (GUABILAB, Porto Alegre, Brazil) and water ad libitum. Rats were maintained on 12-h light-dark cycle.

Tissue Preparation. Females with a mean weight of 260 g were caged with males overnight. On day 21.5 of gestation, the fetuses were delivered by rapid hysterectomy after decapitation of the mother, then wiped, and the umbilical cord was cut. The operations were carried at 37°C, the fetuses were killed by decapitation and their brains were quickly removed and slices prepared in a total time of about 3 min. The rats used at the other ages were also killed by decapitation. The cerebral cortices were cut into 0.3-mm slices using a MacIlwain tissue chopper. The protocol was 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.

Incubation System. For the measurement of protein synthesis, lipid synthesis, and CO_2 production, between 40–50 mg of cerebral cortex slices were incubated in 1) 1.5 ml Krebs-Ringer bicarbonate buffer pH 7.4 (KRb) containing 0.2 mM L-leucine + 0.3 μCi L-[U-^{14}C]leucine or 2) 1.5 ml KRb containing 0.2 mM L-leucine + 0.3 μCi L-[U-^{14}C]leucine + the energetic nutrient utilized in the following concentrations: D-glucose 5.0 mM; D-mannose 5.0 mM; D-fructose 10.0 mM; L-lactate 10.0 mM; D-β-hydroxybutyrate 2.0 mM; L-glutamine 2.0 mM; glycerol 1.0 mM. Incubations were carried out in flasks after contents were gassed with a 95% O_2–5% CO_2 mixture for 1 min and then sealed with rubber caps. The slices of cerebral cortex were incubated at 35°C for 1 h in a Dubnoff metabolic shaker (60 cycles/min) according to the method of Dunlop et al. (19). The remaining procedures for the determination of the radioactivity incorporated to CO_2 and lipids were performed according to Govinatzki et al. (20). Incubation was stopped by adding 0.25 ml 50% TCA through the rubber cap. Then 0.25 ml of 1 M hyamine hydroxide was injected into the central wells. The flasks were shaken for further 30 min at 35°C to trap CO_2. After, 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 and transferred to tubes. After centrifugation, the precipitate was washed three times with 10% TCA, and lipids were extracted with chloroform-methanol (2:1). The chloroform-methanol phase was evaporated in vials and radioactivity was measured. The precipitate resulting after washing with chloroform: methanol (2:1) was dissolved in concentrated formic acid and radioactivity was measured. This radioactivity represents protein synthesis from amino acids.

All results were expressed considering the initial specific activity of the incubation medium. Here, we denominated *lipid synthesis* the rate of incorporation of ^{14}C derived from labeled substrate into the lipid fraction. All results were 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.

Statistical Analysis. Data were analyzed statistically by ANOVA and by the Duncan multiple-range test, with the level of significance set at $P < 0.05$.

RESULTS

Table I shows the ontogenetic changes in basal amino acids metabolism. CO_2 production from [U-^{14}C]leucine and [1-^{14}C]glycine increased with age. Lipid synthesis from [U-^{14}C]leucine and protein synthesis from [1-^{14}C]glycine decreased with age. Protein synthesis from [U-^{14}C]leucine initially increased and later decreased with age.

Tables II, III, and IV show the effects of nutrients on [U-^{14}C]leucine and [1-^{14}C]glycine metabolism at 21.5 days of gestation and at 10 postnatal days and at adulthood, respectively.

In rats with 21.5 days of gestation (Table II), glucose increased the CO_2 production as well as lipid and protein synthesis from [U-^{14}C]leucine; β-OH butyrate increased lipid synthesis and lactate increased lipid and protein synthesis from the same amino acid. Glucose increased protein synthesis and lactate decreased CO_2 production from from [1-^{14}C]glycine.

In rats at 10 postnatal days (Table III), glucose, mannose and fructose increased CO_2 production as well as lipid (except for fructose) and protein synthesis from [U-^{14}C]leucine; β-OH-butyrate decreased lipid synthesis and glutamine decreased both CO_2 production and lipid synthesis from the same amino acid. Glucose and mannose increased protein synthesis from [1-^{14}C]glycine, fructose increased and β-OH-butyrate

Table I. Ontogenic Changes on Basal L-[U-^{14}C]Leucine and [1-^{14}C]Glycine Metabolism in Cerebral Cortex of Rats

Labeled amino acids	Age	CO_2	Lipid	Protein
L-[U-^{14}C]Leucine	21.5 gestational	25.0 ± 3.1*	10.1 ± 1.4	6.8 ± 0.9*
	10 postnatal days	40.2 ± 3.0	10.6 ± 1.5	12.0 ± 1.6*
	Adult	42.0 ± 3.6	3.8 ± 0.5*	1.5 ± 0.4*
[1-^{14}C]Glycine	21.5 gestational	29.8 ± 2.7*	3.6 ± 3.0	10.6 ± 1.2*
	10 postnatal days	224.0 ± 32.0*	8.7 ± 0.9*	5.6 ± 1.1*
	Adult	101.0 ± 25.3*	3.4 ± 0.6	0.9 ± 0.1*

Note: Results presented as mean ± SD of pmol of amino acids oxidized to CO_2, converted to lipids, or incorporated to protein per mg tissue per h. n = 6 in each group.
For L-[U-^{14}C]leucine, *differ from all other groups ($P < 0.01$) in the same column.
For [1-$_{14}$C]glycine, *differ from all other groups ($P < 0.01$) in the same column.

decreased lipid synthesis, and glutamine decreased both CO_2 production and lipid synthesis from the same amino acid.

In adult rats (Table IV), glucose and mannose increased protein synthesis from [U-^{14}C]leucine; glutamine and lactate decreased CO_2 production, and lactate decreased lipid synthesis from the same amino acid. Glucose increased CO_2 production and protein synthesis from [1-^{14}C]glycine; mannose increased protein synthesis, fructose decreased lipid synthesis and increased protein synthesis, lactate and glutamine decreased CO_2 production from the same amino acid.

It is important to emphasize that at least some observed results concerning the influence of energetic nutrients on amino acids metabolism could be attributed, at least partially, to the isotopic dilution of the radioactive products measured.

DISCUSSION

The effects of ontogeny on leucine and glycine metabolism in rat cerebral cortex (Table I) are in agreement with Dunlop and Lajtha (19) and Patel and Owen (16) that have shown a decrease in protein synthesis with age, and lipid and protein synthesis decrease enormously from seven postnatal day until 3 months from leucine, respectively; Patel and Owen (16) also showed that L-[U-^{14}C]leucine oxidation to CO_2 increases with age. Indeed, L-[1-^{14}C]leucine oxidation to CO_2 is much higher than its incorporation to protein in cerebral cortex of adult rats (15). This result was also found by Patel and Owen (16), using L-[U-^{14}C]leucine as precursor, which is similar to our results (Table I).

The [1-^{14}C]glycine oxidation to CO_2 showed an increase of 7 times at 10 postnatal days of age compared to fetal (Table I). This clear increase was prob-

Table II. Effect of Different Energy Nutrients on L-[U-^{14}C]Leucine and [1-^{14}C]Glycine Metabolism in Cerebral Cortex of Rats at 21.5 Days of Gestation

Labeled amino acids	Addition (group no.)	CO_2	Lipid	Protein
L-[U-^{14}C]Leucine	None (1)	25.0 ± 3.1	10.1 ± 1.4	6.8 ± 0.9
	Glucose 5.0 mM (2)	30.2 ± 2.5*	37.6 ± 2.7*	13.0 ± 1.6*
	β-OH 2.0 mM (3)	29.8 ± 2.8	27.6 ± 2.0*	6.1 ± 0.7
	Lactate 10.0 mM (4)	25.6 ± 2.0	26.0 ± 1.6*	10.0 ± 1.8*
[1-^{14}C]Glycine	None (5)	29.8 ± 2.7	3.6 ± 3.0	10.6 ± 1.2
	Glucose 5.0 mM (6)	28.4 ± 2.9	3.6 ± 3.0	17.2 ± 1.4*
	β-OH 2.0 mM (7)	25.0 ± 2.2	3.0 ± 3.2	12.4 ± 1.5
	Lactate 10.0 mM (8)	20.6 ± 2.4*	3.2 ± 3.1	10.0 ± 1.4

Note: Results presented as mean ± SD of pmol of amino acids oxidized to CO_2, converted to lipids, and incorporated to protein per mg tissue per h. n = 6 in each group.
*$P < 0.01$ in comparison with basal condition (none).

Table III. Effect of Different Energy Nutrients on L-[U-^{14}C]Leucine and [1-^{14}C]Glycine Metabolism in Cerebral Cortex of Rats at 10 Days Postnatal

Labeled amino acids	Addition (group no.)	CO_2	Lipid	Protein
L-[U-^{14}C]Leucine	None (1)	40.2 ± 3.0	10.6 ± 1.5	12.0 ± 1.6
	Glucose 5.0 mM (2)	70.4 ± 4.1*	34.6 ± 3.8*	30.0 ± 3.5*
	Mannose 5.0 mM (3)	65.8 ± 6.4*	37.8 ± 3.8*	36.2 ± 3.0*
	Fructose 10.0 mM (4)	49.0 ± 3.0*	10.2 ± 1.3	38.5 ± 3.7*
	β-OH 2.0 mM (5)	35.0 ± 3.2	6.4 ± 1.0*	14.0 ± 2.1
	Lactate 10.0 mM (6)	38.6 ± 3.5	10.3 ± 1.5	19.4 ± 2.5
	Glutamine 2.0 mM (7)	17.0 ± 1.9*	6.7 ± 1.5*	8.7 ± 1.5
	Glycerol 1.0 mM (8)	32.0 ± 3.9	8.0 ± 1.4	12.2 ± 2.2
[1-^{14}C]Glycine	None (1)	224.0 ± 32.0	8.7 ± 0.9	5.6 ± 1.1
	Glucose 5.0 mM (2)	235.0 ± 38.7	9.8 ± 1.6	18.0 ± 3.5*
	Mannose 5.0 mM (3)	221.0 ± 25.6	10.7 ± 1.8	11.0 ± 2.3*
	Fructose 10.0 mM (4)	230.0 ± 35.4	11.6 ± 1.8*	6.4 ± 1.2
	β-OH 2.0 mM (5)	230.8 ± 25.1	6.2 ± 1.4*	5.1 ± 0.8
	Lactate 10.0 mM (6)	233.6 ± 25.7	8.6 ± 1.5	4.0 ± 0.7
	Glutamine 2.0 mM (7)	174.0 ± 22.2*	6.7 ± 1.1*	6.2 ± 0.8
	Glycerol 1.0 mM (8)	234.2 ± 27.4	8.4 ± 1.1	7.0 ± 1.3

Note: Results presented as mean ± SD of pmol of amino acids oxidized to CO_2, converted to lipids, and incorporated to protein per mg tissue per h. n = 6 in each group
For L-[U-^{14}C]leucine, *differ from basal groups ($P < 0.01$).
For [1-^{14}C]glycine, *differ from basal groups ($P < 0.01$).

ably due to a rise in the astrocyte/neuron proportion at 10 days, enhancing the astrocyte glycine cleavage system, the main pathway of glycine oxidation (21,22). We have shown that the production of $^{14}CO_2$ from [1-^{14}C]glycine was thirty times higher than $^{14}CO_2$ production from [2-^{14}C]glycine in cerebellum slices of 10-day-old rats, showing that glycine oxidation in CNS by another route than the glycine cleavage system is insignificant (23). The decrease in glycine oxidation in adult age compared to 10 postnatal days may be due to other parameters than the modification of the ratio astrocyte/neuron. As can be observed in Table I, lipid synthesis from glycine showed the highest value at 10 postnatal days, and at adulthood returned to the

Table IV. Effect of Different Energy Nutrients on L-[U-^{14}C]Leucine and [1-^{14}C]Glycine Metabolism in Rat Cerebral Cortex at Adulthood

Labeled amino acids	Addition (group no.)	CO_2	Lipid	Protein
L-[U-^{14}C]Leucine	None (1)	42.0 ± 3.6	3.8 ± 0.5	1.5 ± 0.4
	Glucose 5.0 mM (2)	45.0 ± 3.8	4.5 ± 0.4	3.2 ± 0.4*
	Mannose 5.0 mM (3)	36.0 ± 5.0	3.6 ± 0.6	2.7 ± 0.4*
	Fructose 10.0 mM (4)	42.0 ± 3.6	4.2 ± 0.6	2.4 ± 0.4
	Glutamine 2.0 mM (5)	27.0 ± 3.8*	3.6 ± 0.6	1.7 ± 0.3
	Lactate 10.0 mM (6)	10.0 ± 1.4*	3.1 ± 0.3*	1.8 ± 0.3
[1-^{14}C]Glycine	None (1)	101.0 ± 25.3	3.4 ± 0.6	0.9 ± 0.1
	Glucose 5.0 mM (2)	130.0 ± 23.2*	3.3 ± 0.4	1.7 ± 0.34*
	Mannose 5.0 mM (3)	110.0 ± 19.0	3.4 ± 0.5	1.2 ± 0.2*
	Fructose 10.0 mM (4)	120.0 ± 22.6	2.2 ± 0.4*	1.5 ± 0.2*
	Lactate 10.0 mM (5)	74.0 ± 16.7*	3.2 ± 0.6	0.9 ± 0.1
	Glutamine 2.0 mM (6)	75.0 ± 9.7*	3.4 ± 0.5	0.8 ± 0.1

Note: Results presented as mean ± SD of pmol of amino acids oxidized to CO_2, converted to lipids, and incorporated to protein per mg tissue per h. n = 5 in each group.
For L-[U-^{14}C]leucine, *differ from basal groups ($P < 0.01$).
For [1-^{14}C]glycine, *differ from basal group ($P < 0.05$).

value of fetal age. The increase in lipid synthesis from glycine, at the age of 10 days, is certainly due to the higher synthesis of phospholipids and sphingomyelin since glycine is more converted to serine through the serine hydroxilase than to pyruvate, and ultimately to acetilCoA.

At fetal age, leucine oxidation was stimulated by glucose, whereas lactate decreased glycine oxidation (Table II). The stimulation of CO_2 production could be explained by Krebs cycle reinforcement, whereas the decrease in glycine oxidation to CO_2 by lactate, is hardly explained, because it is oxidized almost exclusively by cleavage system, without the involvement of the Krebs cycle. A decrease in the specific activity is unlikely, because the oxidation velocity to CO_2 is linear until the 1.0 mM (data not shown). At 10 postnatal days (Table III), glucose, mannose and fructose stimulated leucine oxidation and glucose and mannose stimulated its conversion to lipids. The glutamine provoked a decrease in leucine and glycine oxidation. Yudkoff et al. (18) using [^{15}N]leucine as marker, showed that approximately one third of NH_2 group of glutamine is originated from leucine by transamination and the addition of glutamine to the incubation medium possibly decreases the formation of α-keto-isocaproate and further oxidation to CO_2. Sonnewald et al. (24) and McKenna et al. (25) showed that the glutamate concentration regulates its metabolism in astrocytes: at 0.1 mM it is mainly converted to glutamine, whereas in a concentration higher than 0.2 mM it is primarily oxidized in Krebs cycle and converted to lactate. In this study, glutamine was used in saturating concentration, in which probably the main glutamate pathway (coming from glutamine) is the Krebs cycle and its conversion to lactate, with further utilization by neurons. Then, the provision of α-ketoglutarate to Krebs cycle from glutamine can contribute to the decrease of leucine oxidation to CO_2 by glutamine. Indeed, glutamine could slow down glycine oxidation through an increase in NADH concentration, because it occurs almost completely by its cleavage system. Tildon and Roeder (7) showed that L-[U-^{14}C]glutamine oxidation by cerebral cells increases with the animal age, which is in agreement with our previous results (23).

At adulthood, glucose, fructose, and mannose did not affect leucine oxidation (Table IV), whereas glutamine and lactate decreased it. Patel and Owen (16) have shown that glucose has no effect on leucine oxidation to CO_2 in cerebral cortex of adult rats. The decrease in glycine oxidation by glutamine could have the same explanation at 10 days. The inhibition of glycine oxidation by lactate could probably be due to an increase in NADH concentration, because lactate was more oxidized at adulthood.

As shown in Table II, the three energy nutrients utilized increased lipid synthesis from [U-^{14}C]leucine in cerebral cortex of rats with 21.5 days of gestation. Although glucose might stimulate lipid synthesis producing NADPH through pentose cycle and providing glycerol-P for glycerolipid synthesis, we do not have a pertinent explanation for the increase in lipid synthesis by lactate or β-OH-butyrate. In cerebellum slices, we have previously demonstrated that lipid synthesis from [1-^{14}C]glycine and [2-^{14}C]glycine was similar and that there was no synthesis of neutral lipids. Indeed, almost all formed lipids presented an R_f compatible with glycerolipids and sphyngolipids (23).

In rat cerebral cortex at 21.5 days of gestation, the stimulatory effect of glucose on protein synthesis from [U-^{14}C]leucine and [1-^{14}C]glycine (Table II) was certainly not due to the difference in ATP production secondary to the oxidation of the different energy nutrients by CNS, since Bueno et al. (1) showed that the oxidation of lactate to CO_2 in rat cerebellum slices at 21.5 days of gestation was markedly higher than glucose oxidation. The same results were demonstrated by Vicario et al. (2) in brain cells of rats at 21.5 days of gestation: lactate oxidation to CO_2 was higher than βOH-butyrate and glucose. Accordingly, Wada et al. (26) demonstrated that in hippocampus slices of 4-day-old guinea pigs, lactate and β-OH-butyrate preserved ATP levels and neural activity.

Tables III and IV show that glucose, mannose and fructose stimulated protein synthesis from [U-^{14}C] leucine in rat cerebral cortex at 10 days postnatal and at adulthood. These hexoses present a common metabolic route from fructose-6-P; additionally, fructose-6-phosphate can be isomerized to glucose-6-P, and therefore any intermediate of glycolysis pathway between glucose-6-P and fructose-1,6-P could be responsible for the stimulation of protein synthesis. This assumption is based on the fact that glycerol produces glyceraldehyde-3-P and diOH-acetone-P and did not stimulate protein synthesis. Protein synthesis from [U-^{14}C]leucine and [1-^{14}C]glycine was stimulated by the addition of glucose, mannose and fructose (Tables III and IV); in gestational age, lactate stimulated this metabolic pathway from leucine (Table II). Bueno et al. (1) showed a higher lactate oxidation than glucose, in cerebellar slices of rats. The mechanism of lactate stimulation on protein synthesis, at gestational age, was probably different from all other studied ages. Medina and Vicario (27) have demonstrated that lactate is the

main energetic substrate to the CNS in the first 2 h after birth, where the concentration of lactate and glucose is about 10 mM and 1.5 mM, respectively (28). Schurr et al. (29) evidenced that lactate was capable of maintaining neural activity in hippocampus slices of rats for 60 minutes after the addition of iodoacetic acid.

All together, our results show an ontogenetic effect on [U-^{14}C]leucine and [1-^{14}C]glycine metabolism in slices of cerebral cortex of rat, and its modulation by glucose, mannose, fructose, β-OH-butyrate, glycerol, and glutamine on its oxidation to CO_2, on its conversion to lipids and on its incorporation to proteins.

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