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Developmental Effects of Intrauterine Growth Retardation on Cerebral Amino Acid Transport

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ABSTRACT. Early restriction of nutrients during the perinatal period of life can modify the development of the mammalian fetus and have marked repercussions on the ontogeny of the CNS. The brain is vulnerable to undernutrition, with delayed morphologic and biochemical maturation leading to impaired functions. The aim of the present investigation was to assess whether modified brain neurotransmitter and amino acid concentrations found in an animal model of intrauterine growth retardation were related to modified blood-brain amino acid transport properties. Four amino acids were tested: alanine and taurine, plus two neurotransmitter precursors, tryptophan and tyrosine. Intrauterine growth retardation was induced by restriction of maternal-fetal blood flow from the 17th d of gestation. Blood-brain transport of these amino acids was measured by i.v. injection of radiolabeled amino acids in 7-d-old, 21-d-old, and 60-d-old intrauterine growth-retarded or control rats. No major statistical differences were revealed either for brain regional transport or between intrauterine growth-retarded animals and controls at any age studied. Transfer coefficients and influxes remained statistically similar for almost all brain regions in both groups. A significant decrease and different time course for amino acid transport with age related to the blood-brain barrier maturation are confirmed in this model. Our results are related to a major role of the blood-brain barrier as a part of mechanisms leading to "brain growth sparing." (*Pediatr Res* 35: 640–648, 1994)

Abbreviations

BBB, blood-brain barrier
IUGR, intrauterine growth retardation or retarded

Early restriction of nutrients during perinatal life may have considerable influence on somatic development and may result in permanent alterations in mammalians (1, 2). Although the brain is protected by several homeostatic mechanisms against major fluctuations in the availability of essential nutrients (3), numerous studies have shown severe impairment of the CNS consecutive to undernutrition (4–7). Perinatal life appears as a vulnerable period of development during which several organs, including brain with some late maturation structures, are sensitive to nutrient supply.

Changes in cerebral concentrations of monoamine neurotransmitters and their metabolites are observed in animals at birth and up to several weeks after undergoing undernutrition during the *in utero* or early period of life (8). Although controversy stil exists regarding the trend of modifications, most authors agre on a marked increase in some neurotransmitters (*i.e.* serotonin dopamine, and norepinephrine) consecutive to perinatal undernutrition (9–12). Amino acid concentrations such as alanine and taurine are also increased before weaning in the cerebellum and parietal cortex of young experimentally blood supply–restricted rats (13). In addition, plasma amino acid profiles, free *versu* bound amino acid fractions, or ratios between neutral and tota neutral plasma amino acid concentrations are modified in undernutrition and consequently can induce changes in cerebra levels (14, 15).

To further understand the origins and effects of such alterations on fundamental cerebral mechanisms, we investigated in the present study the possible modifications of cerebral amin acid transport at the BBB caused by undernutrition. Undernutrition was induced after surgical restriction of blood supply *i utero* by ligating uterine vessels from the 17th d of gestation (16 17). Experimental IUGR in the fetus is performed in the latte part of fetal life, at the end of the period of neuronal proliferation (9). With our IUGR method, which uses experimental animal and controls from the same litter, any nutritional or endocrina imbalance from the mother is excluded, as may occur with th classic protein-deprivation method. This undernutrition mode by blood supply restriction has clinical relevance because symptoms of the abnormality reported for the rat are correlated t situations frequently encountered in neonatology and pediatric (18).

Four amino acids were tested for cerebral transport: alanin and taurine, plus two neurotransmitter precursors, tyrosine an tryptophan. All these amino acids or their metabolites have bee reported to be modified in the brain of IUGR animal.

Cerebral amino acid transport was determined for newborn (d), weaning (21 d), and young adult (60 d) IUGR and contro rats. An account of some of the findings has been presented as preliminary report (19).

MATERIALS AND METHODS

Animals. Adult male and female rats of the Sprague-Dawle strain were obtained from Iffa Credo (L'Arbresle, France). Afte a 1-wk period of adaptation, animals about 2 to 4 mo old wer used for reproduction. Pregnant females after surgical intervention (see below) were allowed to give birth in individual cage Experimental or control rats of both sexes were collected randomly when 7 or 21 d old. Young adults were isolated from th mothers after 28 d, and male or female were housed in cages b group of four to five animals until they reached 60 d of age.

Animals used in all experiments were kept in a temperatur controlled (21°C) and light-controlled (12 h light and 12 h dar room and were given free access to standard rat food (UA Epinay-sur-Orge, France) and water.

Blood supply restriction procedure. Gestational age was established by allowing the female in estrus phase access to the ma

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on a single night. Uterine blood supply was restricted according to the procedure of Wigglesworth (17) as modified by Chanez *et al.* (16). Briefly, on the 17th d of gestation, females were placed in a dorsal position, and laparotomy was performed with the animal under moderate ether anesthesia. Uterine horns were exposed; in the lowest part of only one horn, the principal segment of uterine artery and vein were double ligated, and secondary uterine vascularization remained functional. The opposite horn was left untouched and those fetuses served as control animals. The closer the fetus was to the ligature, the lower its weight. Microassays of pH, Po_2, and Pco_2 in arterial vessels of the horn showed comparable values between sham-operated controls and blood-restricted females. Only a slight decrease for Po_2 was observed after blood restriction, but it did not reach significance (Chanez C, personal observations). This procedure of blood restriction has to be considered as a model of *in utero* undernutrition, with eventually a slight hypoxia associated a few hours after ligation but with no ischemic damage to the fetuses.

All procedures and experimentations were conducted according to the highest standards of ethical guidance and animal care recommended by the French Department of Agricultural Affairs (Decree 87-848).

After birth delivery, litters were arranged to contain eight to 10 newborns including no more than three to four IUGR animals. Both IUGR animals and controls were kept with a mother until weaning to insure normal lactation and diet. Offspring remained with mothers until 28 d after birth because of a 1-wk delay in weaning for IUGR rats compared with control animals. Young rats were weighed at least twice a week. We defined as IUGR those animals with at least 25% weight reduction compared with average weight of the animal of the same age and controls from the same litter. Weight curves as a function of age for controls and IUGR animals have been established in our experiments for Sprague-Dawley strain. We confirmed that IUGR rats rarely recover between birth and adulthood, as previously reported for another strain (20).

Blood brain transport of amino acids. Experiments were performed from 0900 to 1200 h in random order for age, sex, and group (whether IUGR or control rats) to avoid bias caused by animal circadian rhythms. Experimental protocol was designed to contain animals from at least three different litters in a group, with never more than two animals from the same mother. Amino acid transport at the BBB was studied with the i.v. injection technique described previously by Ohno *et al.* (21). During all surgical and injection procedures, animals were anesthetized (Equithesin 3 μL/g) and controlled for physiologic temperature with an anal probe and a heating pad. The right saphenous vein and the left brachial artery of 7-d-old rats were exposed and cannulated under stereomicroscopy with a 30-gauge needle (22). A similar technique was used for the right femoral vein and left femoral artery of 21-d-old and 60-d-old animals with, respectively, a 26-gauge needle or, directly, a 0.96-mm external diameter catheter.

A bolus of physiologic saline (10 mM *N*-2-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid; 5 mM KCl; 1.5 mM $CaCl_2$; 140 mM NaCl; 5 mM glucose; pH 7.40) containing 0.01 μCi/g of ^{14}C-L-alanine or ^{14}C-L-tryptophan and 0.05 μCi/g of tritiated taurine or L-tyrosine in a volume of 50 to 200 μL according to animal age was injected over a period of 5 s into the saphenous or femoral vein. Simultaneously, blood was withdrawn regularly in a heparinized catheter from the brachial or femoral artery to assess radioisotope distribution in the vascular space as a function of time. Because amino acids were injected in pairs (alanine-taurine and tryptophan-tyrosine), the vascular space correction had been previously determined for each IUGR and control group with three to four animals/age with the use of ^{14}C-sucrose under the same experimental conditions. During short transport experiments, ^{14}C-sucrose (saccharose; molecular weight, 342) does not measurably cross the BBB in adult and young animals (21) and is well adapted in transport studies of small molecular weight compounds (23).

Precisely 90 s after starting the injection, animals were killed by decapitation, and blood from the head was sampled in tubes for hematocrit evaluation, radioisotope activities, and determination of plasma amino acid concentrations. Immediately thereafter, the brain was rapidly removed from the skull, and then 13 cerebral structures from the right hemisphere were carefully collected. Samples of blood, plasma, and brain tissue were placed in preweighed polypropylene vials, reweighed, and digested for 1 h in 1 mL of soluene at 56°C. Blood fractions (10 μL) were bleached with 30% hydrogen peroxide (100 μL). After cooling, samples were mixed with liquid scintillation cocktail before β-counting for ^{14}C and ^{3}H, using a duolabeled counting program on a scintillation spectrophotometer (Intertechnic SL 3000, KONTRON, France). Results are expressed in dpm with the use of a computer program.

Transport of amino acids into brain was expressed as transfer coefficients (K_{in}) from the net quantity of tracer taken up into brain during short perfusion as follows:

$$K_{in} = [Q_t - V_v\, C_f]/[C_{pl} \cdot T]\ (\mu L \cdot min^{-1} \cdot g^{-1})$$

where Q_t is the total quantity of tracer in the brain sample (dpm/g), V_v is the vascular volume (mL/g) estimated from the brain distribution volume of ^{14}C-sucrose, C_f is the quantity of tracer (dpm/g) in blood at time of decapitation, C_{pl} represents the averaged concentration of tracer in plasma (dpm/μL) assessed by arterial blood withdrawal by catheter, and T the experimental time in minutes starting from the beginning of injection to animal decapitation. This calculation method for transfer coefficient is valid as long as uptake into brain is linear and unidirectional and transformation into metabolites is negligible. Linear transport has been validated for most tracers over the time range used (24, 25), and short experimental times are considered as limiting factors to metabolization of tracers. Brain influx rates (J_{in}) for amino acids were derived from measured transfer coefficients (K_{in}) and plasma amino acid concentrations (C_p) as follows:

$$J_{in} = K_{in} \cdot C_p\ (nmol \cdot min^{-1} \cdot g^{-1}) \cdot 10^{-3}$$

Plasma amino acid concentrations (C_p) are given in μmol/L (nmol/mL) of plasma, as determined by HPLC.

Plasma amino acid concentrations. Taurine, alanine, tryptophan, and tyrosine concentrations in plasma were determined by the method of Lindroth and Mopper (26) and Lindroth *et al.* (27) and were slightly modified in our laboratory to improve tryptophan separation. After a short centrifugation to remove blood cells, blood samples containing norvaline as an internal standard were deproteinized with a volume of 25% sulfosalicylic acid adjusted to blood volume. The clear protein-free fractions resulting from a second centrifugation were derivatized with O-phthaldialdehyde prepared in potassium borate (1 M; pH 10.4) for neutralization, then subjected to HPLC with fluorescence detection. Separation was performed at room temperature on a reverse-phase column (SuperSpher 100 RP-18, Merck, Nogent sur Marne, France).

Concentrations of the four analyzed amino acids were calculated by the internal standard calculation method, with norvaline added in known amount to every plasma sample. Samples were analyzed at least twice to ensure reproducibility of results.

Materials. All amino acid radioisotopes were purchased from Amersham (Les Ulis, France). ^{14}C-sucrose was obtained from CEA (Gif-sur-Yvette, France). The soluene and toluene scintillator were from Packard (Rungis, France).

All chemical products, including O-phthaldialdehyde (Sigma, St. Louis, MO); sulfosalicylic acid, salts for physiologic saline, buffers, and mobile phases for HPLC gradient (all supplied by Merck, Darmstadt, Germany), were of the highest purity commercially available. The HPLC system consisted of an automatic

AS 4000 autosampler, a fluorescence detector F-1000 spectrofluorimeter, and a D-2500 integrator (all systems supplied by Merck-Clevenot Laboratories, Nogent-sur-Marne, France).

Statistical analysis. Values presented in tables and figures are mean ± SEM. Statistical calculations were performed, after checking variance homogeneity, by either *t* test or analysis of variance with the Bonferroni adjustment for multiple comparisons. Threshold of significance was set for both tests at $p < 0.05$.

RESULTS

Transfer coefficients and influxes at the BBB were analyzed for regional differences among 13 brain regions, differences between IUGR and control rats at 7, 21, and 60 d, and finally for modifications with development from birth to adulthood. Plasma concentrations of the four amino acids studied were also examined for possible alterations between IUGR animals and controls with age.

Before any experiments with amino acids, the vascular space was estimated in each brain structure of interest for IUGR and controls at 7, 21, and 60 d. We did not observe significant modifications between experimental and control groups (Table 1).

Amino acid transfer coefficients. Among all the cerebral structures sampled, few amino acid transfer coefficients presented significant and consistent regional differences for both IUGR and control animals. Only the cerebellum at 7 d exhibited significantly higher transfer coefficients for alanine and tyrosine with IUGR and control animals and for tryptophan solely for the IUGR group. Although the transfer coefficients for taurine in both groups and for tryptophan in controls do not appear statistically different for the cerebellum at the same age, a marked trend to higher transfer coefficients for these two amino acids can also be noticed compared with other structures (Tables 2–5). No regional difference was found for 21-d-old control or IUGR animals.

Young adults (60 d) presented lower transport values for alanine in the thalamus of controls and higher transport values for taurine in the hypothalamus of both IUGR rats and controls.

Comparisons between IUGR rats and controls, whatever the age, do not show alterations in amino acid transfer coefficients, except for alanine in 60-d-old controls and IUGR rats, which is significantly modified for olfactory bulb, striatum, and superior colliculus.

During development, a marked decrease in blood-brain transport occurred for all amino acids and for both IUGR and cont animals. However, the trend varied depending on the amino a and cerebral structure. The transfer coefficients followed a g eral decline but with different time courses. Thus, the trans coefficient for alanine was slightly decreased between 7 and 2 of life and then suddenly fell rapidly between 21 and 60 d (3 decrease between 7 and 21 d and 72% between 21 and 60 d the hippocampus). In contrast, taurine showed a marked decre between d 7 and 21 (more than 60% in the hippocampus) a just a slight decrease subsequently (only 25% in the same stru ture). Tryptophan seemed to observe a more regular trend, w no sudden modifications with age. In contrast, tyrosine decrea markedly from weaning to the adult age. In fact, because tr tophan and tyrosine share the same carrier at the BBB w similar Km or higher for tyrosine, in theory comparable trans coefficients—or higher for tryptophan—should be reported both amino acids at least for the adult group.

Plasma amino acid concentrations. Except for higher alan concentrations in IUGR plasma at 7 d, no significant differen between pathologic and control groups was observed at any a for any of the amino acids assayed (Figs. 1 and 2).

Throughout development, we observed different patterns w a general trend to lower plasma amino acid concentrations young adults. Indeed, tyrosine was about 80% lower in ac than in newborn plasma, with a 60% or more decrease occurr between d 7 and 21. A comparable although less marked tr occurred for the other neurotransmitter precursor, tryptoph with a pronounced 40% drop between d 7 and 21 and a plat or a slight increase from weaning to adulthood. Alanine increa between d 7 and 21 for control animals and then declined u d 60. A similar phenomenon occurred for IUGR between d and 60, but a plateau occurred before weaning with a significa higher concentration at 7 d. Taurine plasma concentration the only amino acid plasma value not affected during devel ment.

All plasma amino acid concentrations are comparable to th that have been reported in the literature when previously scribed (16, 20, 28).

Amino acid influx. The parietal cortex, hippocampus, a cerebellum, where amino acid and monoamine concentrati have previously been reported to be modified in IUGR (were chosen as representative brain structures. Like tran coefficients, influxes did not exhibit regional differences am cerebral structures or major alterations between IUGR and c trol animals. Although newborn cerebellum had higher inf

Table 1. *Vascular space in IUGR and control animals**

	7 d after birth		21 d after birth		60 d after birth	
	IUGR	Control	IUGR	Control	IUGR	Control
OB	48.61 ± 5.07	42.12 ± 9.67	42.42 ± 4.68	47.10 ± 3.12	52.73 ± 7.16	44.72 ± 4.12
Hy	14.45 ± 1.11	12.16 ± 2.43	19.38 ± 2.10	20.58 ± 1.10	25.11 ± 3.15	26.05 ± 3.36
PC	10.75 ± 0.64	12.13 ± 3.48	13.79 ± 0.86	12.99 ± 1.25	17.42 ± 1.53	19.87 ± 0.73
FC	18.26 ± 1.22	12.64 ± 3.05	22.30 ± 1.87	24.48 ± 2.61	30.59 ± 3.91	29.09 ± 5.04
OC	24.28 ± 2.57	15.09 ± 3.02	19.80 ± 1.68	19.75 ± 1.52	27.90 ± 2.40	29.71 ± 6.05
S	8.40 ± 0.47	7.13 ± 1.89	8.58 ± 0.32	15.55 ± 4.39	12.36 ± 1.32	12.65 ± 0.98
Hi	18.14 ± 4.08	9.98 ± 2.14	12.18 ± 0.83	15.23 ± 1.50	17.36 ± 1.64	18.77 ± 1.89
T	13.66 ± 1.33	10.06 ± 2.32	13.82 ± 2.07	23.86 ± 6.59	19.05 ± 2.12	20.62 ± 1.54
M	13.18 ± 0.50	10.30 ± 1.80	17.11 ± 0.21	18.99 ± 2.40	24.54 ± 2.97	25.26 ± 2.42
CS	14.01 ± 0.51	10.89 ± 2.50	18.47 ± 3.67	18.88 ± 1.41	25.83 ± 4.19	29.92 ± 4.44
CI	16.05 ± 1.00	10.53 ± 2.09	24.14 ± 1.88	24.72 ± 3.27	29.89 ± 1.83	40.21 ± 4.07
PM	18.67 ± 2.40	11.02 ± 1.91	21.38 ± 0.58	18.46 ± 1.76	27.53 ± 3.76	29.67 ± 4.92
Cerb	26.75 ± 3.87	18.70 ± 1.66	22.96 ± 1.98	21.59 ± 1.37	28.80 ± 2.42	31.44 ± 3.66

* Each value is the mean ± SEM of three to four individual determinations in the IUGR and control groups. Vascular space as calculated ^{14}C-sucrose in independent experiments in μL/g of fresh tissue in IUGR and control animals for 13 cerebral structures: olfactory bulbs (hypothalamus (Hy), parietal cortex (PC), frontal cortex (FC), occipital cortex (OC), striatum (S), hippocampus (Hi), thalamus (T), midbrain colliculus superior (CS), colliculus inferior (CI), pons-medulla (PM), and cerebellum (Cerb). Statistical analysis was performed with *t* test to com regional differences and values between IUGR animals and controls (*n* = three to four animals per group). No significant difference was obse ($p < 0.05$).

Table 2. *Time course of alanine transfer coefficient in IUGR and control animals**

	7 d after birth		21 d after birth		60 d after birth	
	IUGR	Control	IUGR	Control	IUGR	Control
OB	38.3 ± 5.3^{a}	42.2 ± 3.5^{a}	ND	24.6 ± 2.1^{b}	6.3 ± 1.1^{b}	11.4 ± 1.4^{c}†
Hy	30.0 ± 2.5^{a}	34.5 ± 2.3^{a}	25.7 ± 1.6^{a}	25.8 ± 1.6^{b}	12.3 ± 1.2^{a}	13.4 ± 1.5^{c}
PC	46.6 ± 5.3^{a}	47.9 ± 3.5^{a}	30.7 ± 3.3^{b}	30.6 ± 2.6^{b}	8.6 ± 0.6^{c}	8.5 ± 0.5^{c}
FC	43.6 ± 7.8^{a}	57.6 ± 5.7^{a}	29.3 ± 3.6^{a}	26.8 ± 1.5^{b}	8.3 ± 0.9^{b}	10.7 ± 0.7^{c}
OC	43.4 ± 5.0^{a}	49.9 ± 4.9^{a}	28.4 ± 3.2^{b}	30.3 ± 2.8^{b}	9.3 ± 0.5^{c}	11.6 ± 0.4^{c}
S	28.4 ± 3.8^{a}	35.5 ± 3.6^{a}	29.5 ± 3.4^{a}	24.9 ± 1.8^{b}	6.9 ± 0.9^{b}	10.0 ± 0.8^{c}†
Hi	38.8 ± 4.5^{a}	49.7 ± 4.8^{a}	26.9 ± 3.3^{b}	26.1 ± 0.5^{b}	8.9 ± 2.1^{c}	8.4 ± 0.8^{c}
T	35.6 ± 3.2^{a}	40.9 ± 5.3^{a}	30.5 ± 4.2^{a}	23.7 ± 1.9^{b}	7.2 ± 0.5^{b}	5.9 ± 0.6^{c}‡
M	30.9 ± 3.1^{a}	33.5 ± 3.3^{a}	29.2 ± 3.7^{a}	27.0 ± 2.5^{a}	7.6 ± 0.6^{b}	7.8 ± 0.7^{b}
CS	33.9 ± 4.8^{a}	36.5 ± 3.9^{a}	28.1 ± 3.6^{a}	27.6 ± 2.5^{a}	9.2 ± 0.5^{b}	7.1 ± 0.3^{a}†
CI	34.8 ± 4.3^{a}	37.9 ± 3.5^{a}	31.8 ± 3.6^{a}	34.0 ± 3.2^{a}	9.4 ± 0.8^{b}	9.8 ± 0.9^{a}
PM	37.3 ± 4.9^{a}	50.5 ± 3.8^{a}	35.9 ± 4.0^{a}	36.4 ± 2.8^{b}	13.1 ± 0.8^{b}	13.3 ± 1.1^{c}
Cerb	69.2 ± 7.7^{a}‡	74.6 ± 6.8^{a}‡	34.1 ± 4.2^{b}	32.2 ± 2.7^{b}	10.1 ± 0.4^{c}	11.4 ± 1.2^{c}

* Each value is the mean ± SEM in μL · min^{-1} · g^{-1} of four to eight individual determinations in the IUGR and control groups. Evolution of amino acid transfer coefficients in IUGR and control animals for 13 cerebral structures: olfactory bulbs, hypothalamus, parietal cortex, frontal cortex, occipital cortex, striatum, hippocampus, thalamus, midbrain, colliculus superior, colliculus inferior, pons-medulla, and cerebellum. See brain region abbreviations in Table 1. Analysis of variance with Bonferonni adjustment for multiple comparisons was used to observe transfer coefficients evolution with age. All values with a different superscript letter are significantly different. A significant threshold of $p < 0.05$ chosen for all statistical analyses. ND, not done.

† Statistical analysis performed with *t* test to compare values between IUGR animals and controls.

‡ Statistical analysis performed with *t* test to compare regional differences.

Table 3. *Time course of taurine transfer coefficient in IUGR and control animals**

	7 d after birth		21 d after birth		60 d after birth	
	IUGR	Control	IUGR	Control	IUGR	Control
OB	15.4 ± 3.7^{a}	16.0 ± 3.2^{a}	5.6 ± 1.1^{b}	ND	4.9 ± 0.8^{b}	6.6 ± 0.7^{b}
Hy	12.7 ± 2.3^{a}	10.3 ± 1.7^{a}	5.8 ± 0.6^{b}	6.6 ± 0.4^{a}	8.4 ± 1.7^{a}†	10.3 ± 1.2^{a}†
PC	11.4 ± 2.6^{a}	8.9 ± 2.1^{a}	5.7 ± 1.1^{a,b}	6.4 ± 0.6^{a}	4.5 ± 0.3^{b}	4.2 ± 0.6^{a}
FC	10.7 ± 2.6^{a}	13.1 ± 2.6^{a}	5.3 ± 0.9^{a,b}	5.5 ± 0.7^{b}	3.9 ± 0.5^{b}	5.4 ± 0.8^{b}
OC	10.9 ± 2.3^{a}	12.6 ± 2.1^{a}	6.0 ± 1.1^{a}	6.5 ± 1.1^{b}	5.3 ± 0.5^{a}	5.0 ± 0.3^{b}
S	6.2 ± 1.4^{a}	6.5 ± 1.1^{a}	4.5 ± 0.8^{a}	3.2 ± 0.5^{b}	3.1 ± 0.4^{a}	2.9 ± 0.3^{b}
Hi	10.0 ± 1.7^{a}	10.2 ± 2.0^{a}	4.3 ± 0.8^{b}	3.7 ± 0.6^{b}	3.1 ± 0.2^{b}	2.9 ± 0.1^{b}
T	10.7 ± 1.7^{a}	9.1 ± 2.1^{a}	5.2 ± 1.2^{b}	3.3 ± 0.7^{b}	3.9 ± 0.5^{b}	4.0 ± 0.08^{a,b}
M	11.3 ± 1.9^{a}	9.0 ± 1.2^{a}	6.0 ± 1.1^{b}	6.3 ± 0.9^{a,b}	4.3 ± 0.4^{b}	4.7 ± 0.2^{b}
CS	11.0 ± 1.5^{a}	10.3 ± 2.0^{a}	5.6 ± 0.6^{b}	6.4 ± 0.9^{a,b}	4.5 ± 0.5^{b}	4.7 ± 0.5^{b}
CI	13.0 ± 2.3^{a}	9.9 ± 1.3^{a}	7.9 ± 1.2^{a,b}	7.9 ± 1.1^{a}	5.6 ± 0.5^{b}	5.9 ± 0.5^{a}
PM	13.9 ± 2.8^{a}	15.7 ± 3.1^{a}	7.5 ± 1.4^{b}	9.4 ± 1.3^{a,b}	5.1 ± 0.6^{b}	6.8 ± 0.3^{b}
Cerb	17.1 ± 2.9^{a}	16.3 ± 2.2^{a}	5.0 ± 0.8^{b}	6.1 ± 0.9^{b}	3.9 ± 0.3^{b}	4.0 ± 0.2^{b}

* Each value is the mean ± SEM in μL · min^{-1} · g^{-1} of four to eight individual determinations in IUGR and control groups. See legend to Table 1 for abbreviations. See legend to Table 2 for explanation of superscript letters.

† Statistical analysis performed with *t* test to compare regional differences.

Table 4. *Time course of tryptophan transfer coefficient in IUGR and control animals**

	7 d after birth		21 d after birth		60 d after birth	
	IUGR	Control	IUGR	Control	IUGR	Control
OB	79.7 ± 9.1^{a}	70.9 ± 11.2^{a}	39.8 ± 6.3^{b}	40.7 ± 8.8^{b}	25.9 ± 3.6^{b}	19.7 ± 2.4^{b}
Hy	52.1 ± 4.6^{a}	47.6 ± 5.1^{a}	35.6 ± 4.9^{b}	39.5 ± 7.2^{a}	22.0 ± 2.1^{b}	16.5 ± 2.8^{b}
PC	73.3 ± 7.1^{a}	63.7 ± 7.6^{a}	46.3 ± 6.9^{b}	50.6 ± 10.1^{a}	22.6 ± 2.4^{c}	16.3 ± 3.0^{b}
FC	77.0 ± 5.1^{a}	72.5 ± 11.0^{a}	40.7 ± 6.1^{b}	44.9 ± 8.9^{a}	21.5 ± 2.4^{c}	15.1 ± 2.5^{b}
OC	78.4 ± 7.1^{a}	75.2 ± 11.2^{a}	45.0 ± 6.5^{b}	50.3 ± 10.4^{a}	23.8 ± 3.2^{c}	15.9 ± 2.1^{b}
S	71.4 ± 7.9^{a}	58.4 ± 7.2^{a}	42.5 ± 5.9^{b}	41.2 ± 8.1^{a}	21.3 ± 2.5^{c}	18.0 ± 2.6^{b}
Hi	72.7 ± 9.1^{a}	71.1 ± 10.5^{a}	39.0 ± 5.4^{b}	43.2 ± 9.5^{a,b}	20.6 ± 2.6^{b}	14.8 ± 2.4^{b}
T	68.8 ± 6.5^{a}	63.6 ± 5.6^{a}	41.7 ± 5.6^{b}	40.6 ± 9.3^{b}	20.2 ± 2.3^{c}	17.6 ± 3.4^{b}
M	51.8 ± 5.6^{a}	45.7 ± 5.0^{a}	45.2 ± 8.1^{a}	42.8 ± 8.8^{a}	19.4 ± 2.1^{b}	14.6 ± 2.6^{b}
CS	57.7 ± 5.5^{a}	51.3 ± 5.2^{a}	46.8 ± 6.5^{a}	52.5 ± 11.2^{a}	22.6 ± 2.4^{b}	17.4 ± 2.8^{b}
CI	56.6 ± 5.8^{a}	56.3 ± 6.5^{a}	45.7 ± 6.7^{b}	57.7 ± 12.1^{a}	24.2 ± 4.0^{b}	17.6 ± 3.1^{b}
PM	73.6 ± 8.1^{a}	67.7 ± 7.2^{a}	44.8 ± 6.5^{b}	49.7 ± 10.1^{a}	21.8 ± 2.4^{c}	17.2 ± 3.5^{b}
Cerb	96.3 ± 7.8^{a}†	86.0 ± 9.5^{a}	42.3 ± 6.1^{b}	47.5 ± 9.7^{b}	23.4 ± 2.4^{b}	17.6 ± 2.7^{c}

* Each value is the mean ± SEM in μL · min^{-1} · g^{-1} of five to nine individual determinations in the IUGR and control groups. See legend to Table 1 for abbreviations. See legend to Table 2 for explanation of superscript letters.

† Statistical analysis performed with *t* test to compare regional differences.

Table 5. *Time course of tyrosine transfer coefficient in IUGR and control animals**

	7 d after birth		21 d after birth		60 d after birth	
	IUGR	Control	IUGR	Control	IUGR	Control
OB	109.5 ± 11.3^a	90.1 ± 11.0^a	84.2 ± 18.5^a	90.8 ± 20.2^a	63.0 ± 5.2^a	59.3 ± 5.4^a
Hy	70.3 ± 4.8^a	68.5 ± 5.8^a	68.6 ± 13.4^a	83.0 ± 17.9^a	44.8 ± 6.2^a	46.5 ± 3.6^a
PC	92.0 ± 7.4^a	78.5 ± 8.2^{a,b}	81.7 ± 15.0^a	101.0 ± 22.6^a	51.0 ± 3.8^a	45.1 ± 4.1^b
FC	98.9 ± 6.3^a	93.0 ± 12.2^a	76.7 ± 15.2^{a,b}	94.0 ± 20.9^a	51.2 ± 3.4^b	42.8 ± 3.4^b
OC	97.4 ± 5.6^a	95.8 ± 12.7^a	80.1 ± 15.6^{a,b}	98.5 ± 24.4^a	52.8 ± 4.3^b	43.9 ± 2.7^a
S	83.9 ± 7.3^a	69.3 ± 7.9^a	75.4 ± 13.5^{a,b}	83.7 ± 18.1^a	48.1 ± 3.8^b	50.7 ± 6.1^a
Hi	88.1 ± 9.3^a	86.1 ± 11.4^a	71.2 ± 13.1^{a,b}	84.7 ± 19.7^a	46.1 ± 3.7^b	40.8 ± 3.1^a
T	86.6 ± 7.3^a	77.1 ± 10.2^a	76.0 ± 13.6^{a,b}	88.7 ± 21.4^a	47.3 ± 3.7^b	45.5 ± 4.1^a
M	69.4 ± 6.7^a	59.8 ± 6.2^{a,b}	80.6 ± 15.9^a	91.8 ± 21.2^a	46.4 ± 3.8^a	41.7 ± 3.0^a
CS	76.3 ± 6.8^a	64.4 ± 6.9^a	85.2 ± 15.9^a	109.4 ± 27.0^a	54.7 ± 4.4^a	51.2 ± 4.1^a
Cl	73.8 ± 5.9^a	71.4 ± 7.9^{a,b}	87.1 ± 16.4^a	117.3 ± 26.5^a	58.4 ± 5.6^a	52.8 ± 4.5^b
PM	95.5 ± 9.0^a	86.1 ± 9.5^{a,b}	87.5 ± 17.1^a	106.0 ± 23.5^a	52.9 ± 4.2^a	49.3 ± 4.3^a
Cerb	121.4 ± 10.5^a†	108.6 ± 11.0^a†	81.1 ± 15.8^b	102.7 ± 23.6^{a,b}	57.0 ± 4.3^b	51.9 ± 4.3^b

* Each value is the mean ± SEM in $\mu L \cdot min^{-1} \cdot g^{-1}$ of five to nine individual determinations in the IUGR and control groups. See legend to T 1 for abbreviations. See legend to Table 2 for explanations of superscript letters.

† Statistical analysis performed with *t* test to compare regional differences.

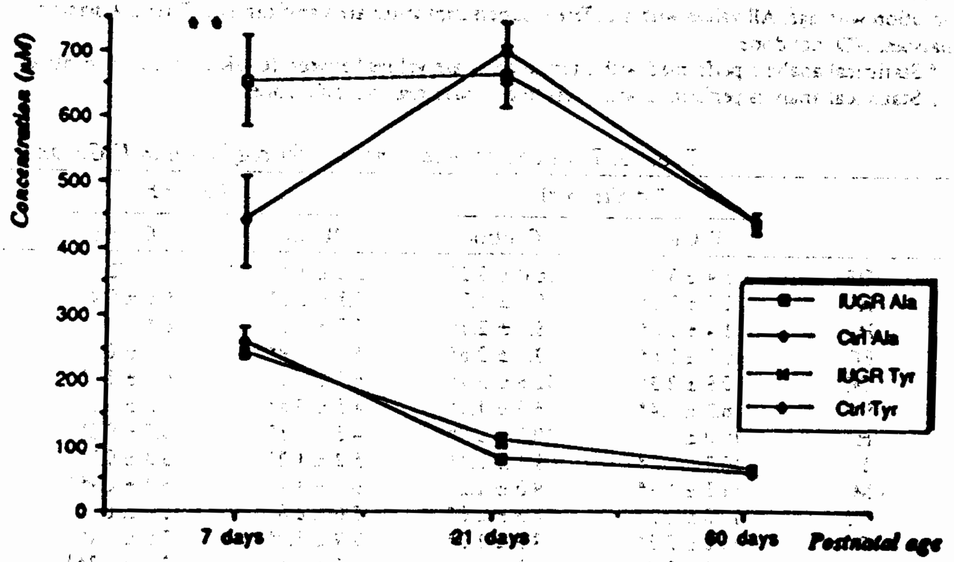

Fig. 1. Plasma alanine and tyrosine trend with age. Developmental changes in plasma amino acid concentrations in IUGR and control (rats. Each point represents the mean ± SEM of nine to 18 independent plasma samples. Amino acid concentrations are given in µM as expr by HPLC determinations. Statistical comparisons are realized according to *t* test: **, $p < 0.05$.

values for most amino acids, these did not reach significant threshold, probably because of heterogeneity in some groups.

The amino acid influx time course observed similar patterns to those described for transfer coefficients. Because plasma amino acid quantification results were similar in control and IUGR animals and because of the clear decreasing trend for transfer coefficients in both groups, a good correlation is logically found between the two transport parameters. Thus, a regular decrease for the influx of the two neurotransmitter precursors, tryptophan and tyrosine, is evident from early life to adulthood (Figs. 3 and 4). Taurine and alanine present differential maturation, with the greatest decrease occuring after weaning for alanine and before this period for taurine (Figs. 5 and 6).

DISCUSSION

The aim of the present study was to investigate blood-brain amino acid transport in IUGR animals during development and also to attempt to correlate our findings with a general mechanism of brain specific protection during the undernutrition state.

IUGR animals exhibit pronounced loss of body weight, at least 25% in our study, with several organs and biochemical factors still presenting highly significant modifications many weeks after undernutrition and often until adulthood. Thus, organs such as the liver, heart, or spleen or brown fatty tis can exhibit more than 50% of weight reduction after unde trition injury (29). The brain seems markedly protected c pared with other structures, with at most a 20% weight reduc and in some cases with physiologic integrity preserved. How although the brain appears well preserved, many biochen parameters still present marked modifications several weeks undernutrition occurs. The brain cholinergic system (30), adrenergic pathways (6), and serotonin and dopamine cer metabolism (9, 10) present marked alterations in concentra or metabolite pathways after severe undernutrition.

In the present investigation, none of the four amino transfer coefficients or influx for any of the 13 cerebral struc showed significant and consistent differences between IUGR control animals at any age considered (Tables 2 through 5 can thus emphasize that the origin of alterations for amino or monoamine concentrations found in some IUGR brai gions (13) is not related to modified influx properties at the of IUGR animals. Nevertheless, we could have expected si cant difference between young IUGR and control animal pecially for tryptophan transfer coefficient and influx. U normal physiologic conditions, approximately 30% of tr phan is free in plasma of 8-d-old rats, whereas after *in*

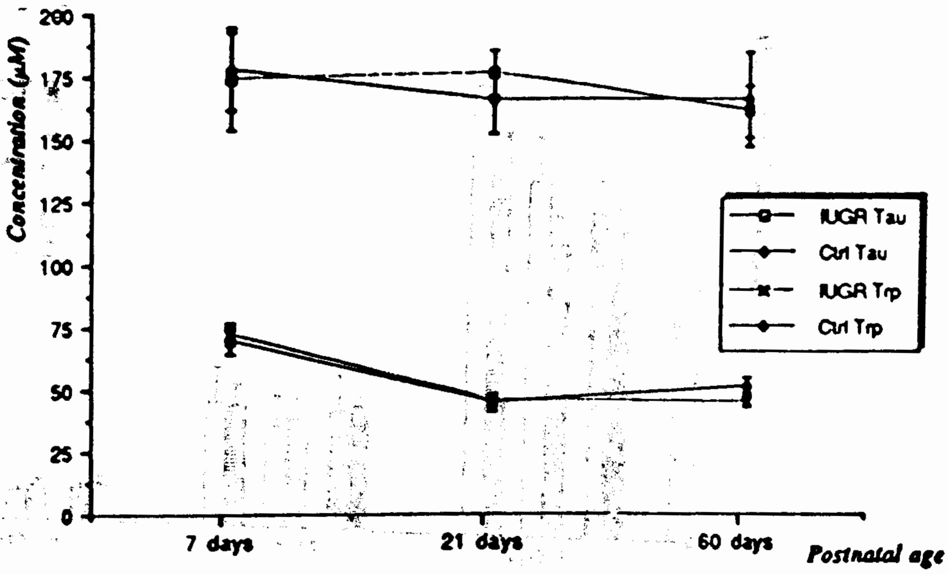

Fig. 2. Plasma taurine and tryptophan trend with age. Developmental changes in plasma amino acid concentrations in IUGR and control (C rats. Each point represents the mean ± SEM of nine to 18 independent plasma samples. Amino acid concentrations are given in μM as expres by HPLC determinations.

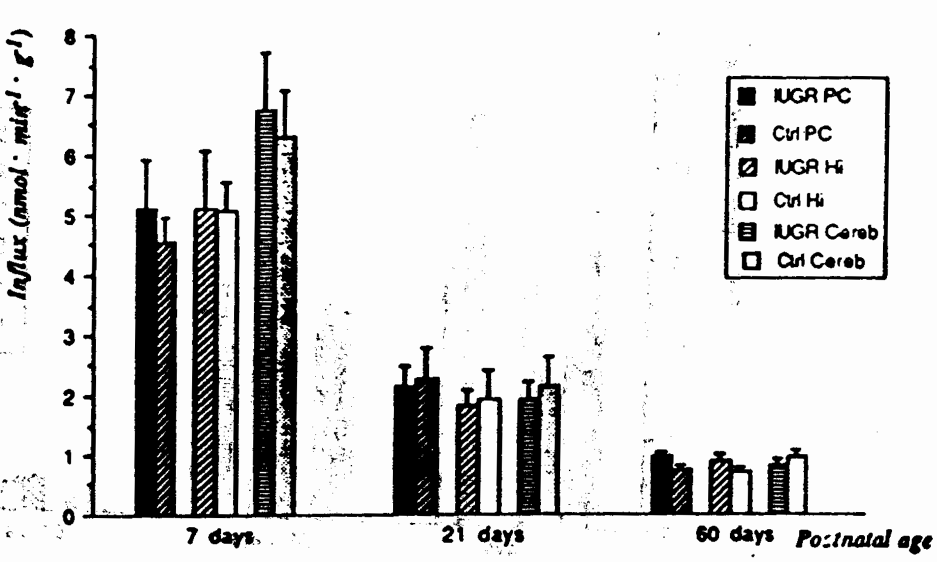

Fig. 3. Tryptophan influx trend with age. Developmental changes in amino acid blood-brain transfer coefficients in IUGR and control (C rats. Three representative brain regions in each group are reported: parietal cortex (*PC*), hippocampus (*Hi*), and cerebellum (*Cereb*). Each value the mean ± SEM of four to nine independent determinations for each age, cerebral structure, and IUGR or control group; *t* test was applied compare IUGR and control cerebral structures, and no difference was statistically significant. Analysis of variance with Bonferonni adjustment multiple comparisons was used to analyze developmental changes with age.

blood supply restriction the free fraction reaches 55% of the total tryptophan (16). If we consider that tryptophan bound to albumin does not cross the BBB, logically such an increase in the free tryptophan fraction should have induced a higher transfer coefficient and influx for young IUGR animals. As reported in Table 4, no statistical difference appears between IUGR and control groups. This result is in accordance with previous studies suggesting that a fraction of tryptophan bound to albumin could participate to blood-brain transfer process (31, 32). As hypothesized, a fraction of bound tryptophan could be readily dissociated in contact with brain microvessels and be available for transendothelial transport (33). Moreover, low cerebral blood flow reported for young rats (34) could markedly enhance tryptophan endothelial transport. Indeed, Smith *et al.* (35) mentioned a higher effective free fraction for tryptophan when perfused *in vivo* at low cerebral blood flow. This factor, along with maturation of neutral amino acid carrier (36), could account for a higher tryptophan transfer coefficient in young animals compared with adults.

Although albumin for young animals does not appear as major restrictive factor to tryptophan transport, throughout c velopment we can observe an important decrease in tryptoph transfer coefficient and influx related to increase in plasr albumin concentration or albumin maturation. Both tryptoph and tyrosine share the same neutral amino acid transporter (system) at the BBB with similar Km and apparent Km or higt for tyrosine (37, 38). When the ratio of transfer coefficie tryptophan/transfer coefficient tyrosine is calculated (Tables and 5), an important decrease—50% or average [Values pressed as "% in average" represent the percentage of variati for the mean of all the brain structures in a group (exce cerebellum) compared with the mean of all the regions (exce cerebellum) in the other group considered]—occurs from bi to adulthood. This decreasing ratio for the two amino ac injected simultaneously tends to confirm that tryptophan bind to albumin plays a restrictive role in tryptophan transfer fr plasma to the brain. Consequently, a higher rate of tryptoph

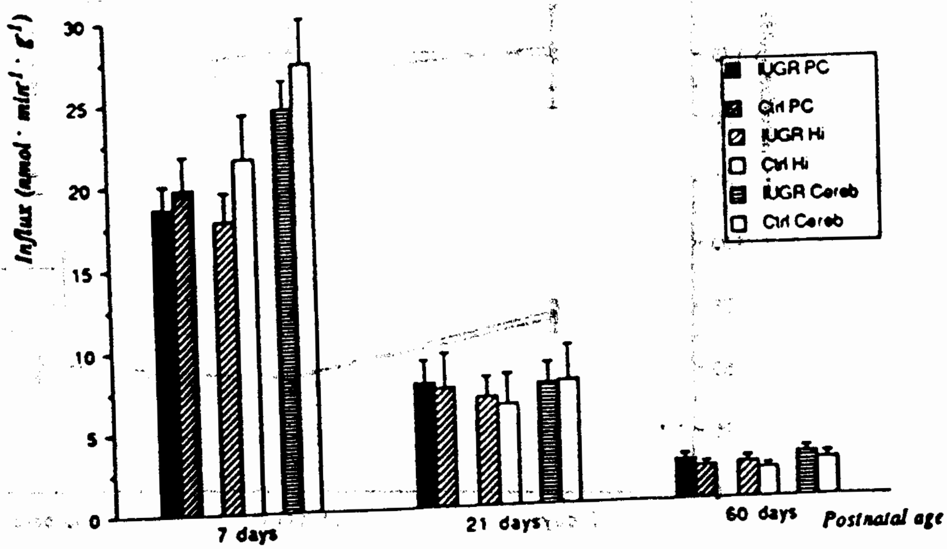

Fig. 4. Tyrosine influx trend with age. See legend to Figure 3 for explanation of data.

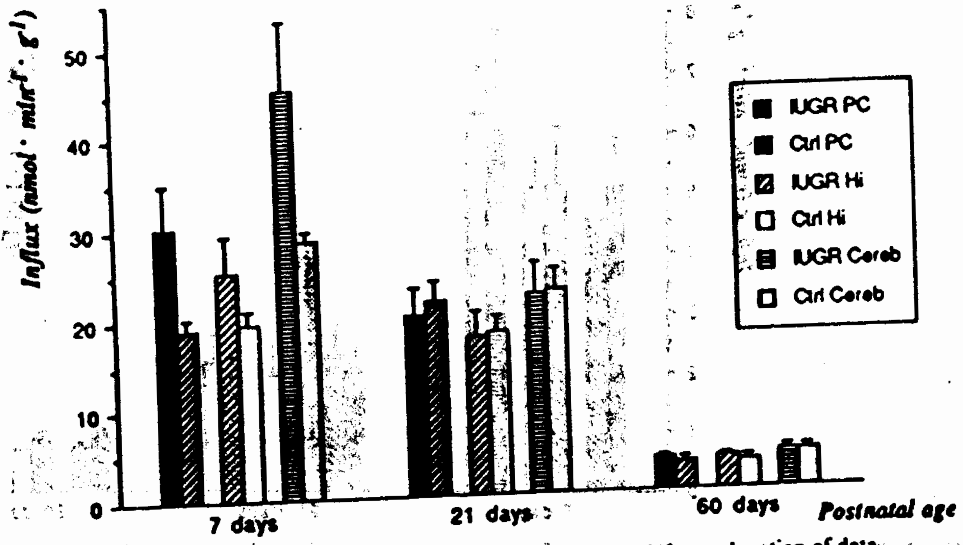

Fig. 5. Alanine influx trend with age. See legend to Figure 3 for explanation of data.

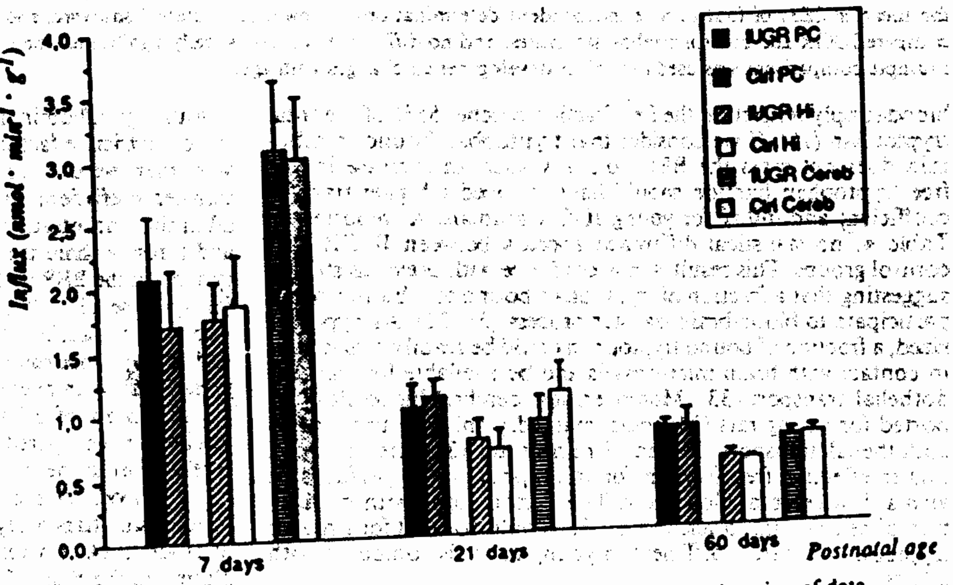

Fig. 6. Taurine influx trend with age. See legend to Figure 3 for explanation of data.

bound to albumin leads in adult plasma to a lower readily exchangeable fraction transportable at the BBB.

Maturation of albumin could also have a major effect on the fraction of tryptophan bound to the protein or on the kinetic of dissociation at the transport site. This parameter could also account for differences in tryptophan transport properties between young and adult groups.

Alanine transfer coefficients for both IUGR and control groups present a relatively slight decrease—28% on average—during the first 3 wk of life, but an important fall-off—67% on average—occurs from weaning to adulthood (Table 2). Nonetheless, alanine influx tends to be increased for 7-d-old IUGR animals, although not significantly. Because influx depends on plasma concentration, this result has to be related to significantly higher plasma alanine (Fig. 1) for young IUGR animals. A decrease in the alanine transfer coefficient is certainly correlated to this amino acid transport system maturation (system alanine, serine, and cysteine), reported to be active for young rats and then reported to lose a preponderant role in alanine transfer along with BBB maturation (39).

In opposition to alanine, taurine transport is mainly affected from birth to weaning with a 49% and 46% on average reduction for IUGR animals and controls, respectively. Then, between weaning and adulthood, only an 18% and 11% average decrease, respectively, occurs with development. Taurine plasma profile and influx are not affected by the IUGR pathologic state. Only brain taurine is modified in IUGR animals, suggesting it could compensate for delayed maturation of prenatally underfed animals.

Interestingly, for both taurine and alanine, efflux from the brain on the abluminal membrane is sodium dependent and probably related to Na^+,K^+-ATPase enzyme activity (40, 41). In addition, Na^+,K^+-ATPase activity is markedly diminished after IUGR (42). It is thus interesting and relevant to correlate a possible decline in amino acid efflux in young IUGR animals compared with controls with the increase in alanine and taurine cerebral concentrations in IUGR animals.

Our entire experimental approach emphasizes protection of the BBB transport mechanisms from irreversible effects subsequent to undernutrition injury. Data obtained in our study are in accordance with the concept of "brain growth sparing" previously observed with the undernutritional state (6). The BBB would to a certain extent play a key role in brain protection against severe decline in nutriment supply. All cerebral structures seem protected by the BBB during undernutrition. Because carriers or transport mechanisms are not affected by undernutrition, the BBB would still ensure normal functions and homeostasis with no major limitation on amino acid transfer rates. Only the cerebellum has a higher transfer coefficient for both 7-d-old IUGR and control rats. This regional difference is certainly related to the delayed maturation reported for the cerebellum (6). Indeed, the cerebellum requires more than 3 postnatal weeks to elaborate its final structures and organization (43) and is certainly more sensitive to undernutrition, as previously reported (8).

On the basis of the present study, we can consider that blood-brain transport does not play any major role in the increased amino acid or monoamine brain concentrations in our model of undernutrition. We cannot affirm that BBB is not sensitive to IUGR. Indeed, we could imagine a possible recovery of the BBB between birth and the 7th d of life or a blood restriction procedure occurring too late in our experimental model after BBB structural maturation. However, at the different ages considered, the structures do not present irreversibly altered transport properties. We can then conclude that amino acid or monoamine modifications previously reported are not due to a hypothetical adaptation of the BBB to the pathologic state. An unmodified BBB could account as a major mechanism leading to "brain growth sparing."

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