PRENATAL IMPAIRMENT OF BRAIN SEROTONERGIC TRANSMISSION IN INFANTS

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Objective To evaluate whether the free fraction of L-tryptophan (L-Trp) and the N1/P2 component of the auditory evoked potentials (AEPs) are associated with impaired brain serotonin neurotransmission in infants with intrauterine growth restriction (IUGR).

Study design We measured free, bound, and total plasma L-Trp and recorded the N1/P2 component of AEP in a prospective, longitudinal, and comparative study comparing IUGR and control infants.

Results Plasma free L-Trp was increased and the amplitude of N1/P2 component was significantly decreased in IUGR relative to control infants. The free fraction of L-Trp and N1/P2 component had a negative association.

Conclusions In newborns with IUGR, the changes in measured plasma free fraction of L-Trp and in the amplitude the N1/P2 component of the AEP suggest an inverse association between free L-Trp and components of the AEP. The changes observed in the free fraction of L-Trp and AEP may be causally associated with brain serotonergic activity in utero. In IUGR, epigenetic factors such as stress-induced disturbances in brain serotonin metabolism or serotonergic activity, identifiable by alterations in AEP, influence cerebral sensory cortex development and may be causally associated with serotonin-related disorders in adulthood. *(J Pediatr 2005;147:592-6)*

In the human brain, serotonergic neurons are present in the fifth week of gestation, and they increase rapidly through the 10th week of fetal life. By week 15, the typical organization of the serotonergic system into the raphe nuclei is complete.[1-5] Evidence exists to support the role of serotonin in the normal process of sensory cortex formation.[6-11] Serotonin depresses or facilitates cortical neuronal activity that is dependent on the type of receptor involved;[12,13] hence it may control gain factors and excitability levels of cortical neurons. Decreased serotonin availability increases neuronal cortical activity in the auditory cortex, which in turn is reflected in the amplitude of the N1/P2 component of auditory evoked potentials (AEPs).[14-16] An opposite effect on the auditory cortex is observed when serotonergic neuronal activity increases. N1 and P2 waveforms recorded from the scalp are AEP components generated by the supratemporal plane of the superior temporal and lateral gyri, and are considered representative of auditory cortex integrative functions.[14-17]

In rats, intrauterine growth restriction (IUGR) leads to increased brain serotonin synthesis accompanied by an increase in the free fraction of plasma L-tryptophan (L-Trp).[18-21] The free fraction of plasma L-Trp and N1/P2 ratio and its components correlate significantly, and they appear to be reliable indicators of changes in serotonergic neurotransmission in rats with IUGR.[16] The normal pattern of the N1/P2 component is disrupted in rats with IUGR.[16] This change in the free fraction of plasma L-Trp has also been detected in human infants with IUGR.[18,22] The purpose of the present study was to test

ACD	Citric acid, sodium citrate, dextrose	FGR	Fetal growth ratio
AEP	Auditory evoked potentials	GABA	γ-amino butyric acid
AgCl	Silver/silver chloride	IUGR	Intrauterine growth restriction
C	Control group	L-Trp	L-tryptophan
Cz	Vertex	MS	Sum of squares minus
EEG	Electroencephalogram	SS	Sum of squares

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Supported by grants from the Mexican Institute of Social Security and CONACYT (FP-0038/740 and 30764-M).

Submitted for publication Sep 13, 2004; last revision received May 11, 2005; accepted Jun 13, 2005.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2005.06.025

the hypothesis that changes in the free fraction of plasma L-Trp and in the N1/P2 component of AEP may be associated and are altered in infants with IUGR. Changes in L-Trp and alterations in N1/P2 waveforms may identify developmental metabolic and cerebrocortical anomalies caused by disrupted serotonergic activity during cerebral cortex differentiation.

METHODS

Subjects

Parents gave consent for their infants to participate in this study. The study group comprised 25 newborns from the service of Neonatology, Gynecology-Obstetrics, Hospital 4, Mexican Institute of Social Security. The group with IUGR included 13 newborns of both sexes with body weight below the 10th percentile of intrauterine growth curves,[23] fetal growth ratio (FGR) < 0.90,[24] and ponderal index of 2.14 ± 0.10.[25] The control group included 12 newborns with body weight between the 10th and 90th percentiles of the same curves, FGR > 0.90, and ponderal index of 2.63 ± 0.06. No clinical signs of other pathologies were observed in either group.

All newborns were breast-fed and also fed with a complement of protein-modified milk diluted to 16% that supplies 20 cal/30 mL, with 3.98 ± 0.81 mg L-Trp/100 mL. Maternal milk and protein-modified milk have similar L-Trp content (FAO Nutrition Studies, Rome 1970, items 375 and 383, Geigy Scientific tables, 7th ed. Basel, Switzerland, Ciba-Geigy, Ltd).[26]

Recording of the N1/P2 Component of the AEPs

Recordings were obtained with one electrode referenced on the vertex (Cz), in a sound-attenuated and electrically shielded room adjacent to the recording apparatus. AgCl electrodes were used (electroencephalogram [EEG] disk electrode NE-101, 10 mm diameter). Binaural 1-KHz tones, lasting 20 ms randomized between 1500 and 2000 ms at 60 dB, were presented in pseudorandomized order through headphones. Data were digitally collected with a sampling rate of 1000 Hz. Artifact rejection was done with 0.1-Hz and 200-Hz filters, from 50 ms prestimulus to 500 ms poststimulus. Fifty sweeps were amplified with a Grass module (preamplifier and amplifier model 7P5, wide-band AC EEG; Star Med, CITY and STATE) with a gain of 10,000 and recorded on computer paper. The *x-y* graphs of the AEPs were examined, and prominent peaks were identified and measured using specific software (multi-lab card with programmable Cain PCL-812P6). Latencies in milliseconds (60 to 120 ms for N1 and 110 to 210 ms for P2) and amplitudes in microvolts (μV) were also calculated. The amplitude of the N1/P2 component of the AEP was considered as the sum of μV's between the crests of the waves N1 and P2.

Biochemical Assays

At 1, 30, and 60 days after birth, 2 mL of blood was collected through venipuncture in borosilicate tubes containing 450 μL of ACD solution (citric acid 9.9 mg, sodium citrate 3.6 mg, and dextrose 11 mg, buffered with Tris acetate 50 mmol, pH 7.4). Blood samples were obtained between 7 and 8 AM and 4 hours after the last feeding, were immediately cooled (to 0 to 4°C) and centrifuged at 500 × *g* in a Sorvall RC5C refrigerated centrifuge. Plasma aliquots were used for the various biochemical assays. An ultrafiltered plasma sample was obtained using Centriflo-Amicon CF50A membranes (Danvers, MA) for the free fraction of plasma L-Trp. The high-performance liquid chromatography fluorescent method of Peat et al[26] was used to quantify the free fraction and total plasma L-Trp. The difference between these 2 was considered to be the fraction bound to albumin.

Table. Clinical data of infants with intrauterine growth restriction and normal controls

	Intrauterine growth restriction n = 13	Controls n = 12
Gestational age (wk)	38.3 ± 0.2	39.5 ± 0.2
Ponderal index	2.14 ± 0.10*	2.63 ± 0.06
Fetal growth ratio	61.8 ± 3.2*	104.6 ± 3.2
Body weight (gm) (days)		
1	1845 ± 93.9*	3246 ± 90.5
30	2544 ± 142.2*	4200 ± 174.4
60	3475 ± 179.0*	5638 ± 143.3
Body length (cm) (days)		
1	44.2 ± 1.0*	49.5 ± 0.9
30	48.3 ± 1.0*	54.0 ± 0.8
60	50.9 ± 1.4*	58.3 ± 1.2

Each point represents the mean value ± SD. Body weight (Treatment: SS = 1340, Df = 5, MS = 268.1. Residual SS = 1340, Df = 5, MS = 5.946). Body length (Treatment: SS = 392.0, Df = 2, MS = 196. Residual SS = 3152, Df = 31, MS = 1.07). Difference were determined by Wilcoxon test and ANOVA.
*p < 0.001.

Data Analysis

Mean values and standard deviations were used for normally distributed data. Differences among mean values were analyzed for significance using Wilcoxon's signed rank test and analysis of variance, with a level significance of $P < .05$.

RESULTS

The IUGR and control infants are described in Table. Free plasma L-Trp concentrations were increased in the IUGR group relative to the control group, confirming previous observations.[18,22] Bound L-trp was decreased, and total L-trp was unchanged (Figure 1).

The group with IUGR demonstrated a significantly decreased amplitude of the N1/P2 component ($P < .05$) (Figure 2) and decreased latencies of P1, N1, and P2 ($P < .05$) (Figure 2). In the control group there was an increase only in N1 latency on day 60, and no changes in P1 and N1 latencies with age were observed (Figure 3).