A Low Brain Serotonergic Neurotransmission in Children With Type 1 Diabetes Detected Through the Intensity Dependence of Auditory-Evoked Potentials

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OBJECTIVE — To determine in children with type 1 diabetes the plasma free fraction of \( \text{L-tryptophan} \) (FFT) and the intensity-dependent auditory-evoked potentials (IDAEPs) as indicators of possible changes in brain serotonergic neurotransmission.

RESEARCH DESIGN AND METHODS — A prospective and comparative study was performed in children with type 1 diabetes and normal control subjects. We measured FFT, bound and total plasma \( \text{L-tryptophan} \), neutral amino acids (NAAs), albumin, free fatty acids (FFAs), glucose, and \( \text{HbA1c} \) (A1C) and recorded IDAEPs with four intensities (40, 60, 90, and 103 dB).

RESULTS — The glycemia, A1C, FFAs, and NAAs in plasma were significantly elevated. The FFT and the FFT-to-total \( \text{L-tryptophan} \) and FFT-to-NAAs ratios were reduced. The latencies of N1 and P2 increased at all intensities and the slope of the amplitude/stimulus intensity function (ASF slope) of the N1/P2 component significantly increased.

CONCLUSIONS — The decrease of the FFT in plasma and increase in the N1/P2 component amplitude may reflect a functional relationship between the brain serotonergic activity with the N1/P2 changes. The increase of the ASF slope in children with type 1 diabetes suggests that the response of the auditory cortex to sound intensity stimulus may be regulated by the serotonergic tone and that decreased serotonergic neurotransmission may provoke a different behavior of sensory cortices. Therefore, the IDAEP (N1/P2 component) may be an electrophysiological indicator of brain changes of serotonergic neurotransmission in children with type 1 diabetes. These changes may be related to psychoemotional manifestations observed in diabetic children such as anxiety and depression.

The study of peripheric markers of the brain serotonergic system in diabetic patients represents an opportunity to evaluate how the metabolic changes due to type 1 diabetes may influence serotonin brain activity. The serotonergic system has a wide distribution in the brain, coming from a small group of multipolar neurons located on the midline of the brainstem. The distribution of the serotonergic neuronal system in the brain of humans and rats has been well described.

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Auditory evoked potentials and type 1 diabetes

tors of these subcomponents. The N1 component of the individual dipole source is measured as the negative peak within 60–120 ms, and the P2 component is measured as the positive peak within 110–210 ms (12–15). So, it is accepted that these components are representative of auditory cortex integrative functions (16).

There is experimental evidence that type 1 diabetes in rats induces a decrease of brain 5-HT synthesis coincident with a lowering of plasma FFT and of brain L-tryptophan together with a long-lasting inhibition of the limiting enzyme tryptophan hydroxylase two (10,11,17) due to a change in its kinetic properties (17). These, in turn, diminish the functional activity of the serotonergic system. Additionally, we described a decrease of FFT that may correspond also to a decreased transport of this amino acid to the brain, with a possible lowering of serotonin synthesis in children with type 1 diabetes (9). Therefore, here we propose the hypothesis that in human children with type 1 diabetes, the FFT in the plasma and IDAEP’s N1/P2 components may also be altered, reflecting brain changes in serotonergic neurotransmission important for the clinical picture of diabetic patients.

**RESEARCH DESIGN AND METHODS** — This study was approved by the ethics committee of the Pediatric Hospital, Xxi Century, National Medical Center, Mexican Institute of Social Security, Mexico City, Mexico. All parents of the patients gave written informed consent after the procedure was fully explained to them. A prospective and comparative study was planned in 23 infants with type 1 diabetes, aged 10.9 ± 0.39 mean ± SD years with a significantly low BMI of 17.71 ± 0.53 kg/m² (Mann-Whitney U-test) (P < 0.01), according to the National Diabetes Group's criteria (18). The second group was made up of 12 nondiabetic children with similar age range 11.25 ± 0.41 years and BMI 20.68 ± 0.59 kg/m² who served as control subjects. No clinical signs of other pathologies were observed in any of the groups in the study.

All infants were fed a normal diet of 55 kcal·kg⁻¹·day⁻¹ (protein 20%, carbohydrates 55%, and lipids 25%). Children with type 1 diabetes were managed with a combination of fast- and intermediate-action insulin, 0.8–1.0 U·kg⁻¹·day⁻¹. Two milliliters of blood were collected by venopuncture in borosilicate tubes containing 450 μl of ACD solution (3.6 mg sodium citrate, 9.9 mg citric acid, and 11.0 mg dextrose, 50 mmol/l buffered with Tris-base; pH 7.4) between 0700 and 0800 and 12 h after the last feeding. The tubes containing the blood samples were immediately cooled (0–4°C) on ice and centrifuged at 500g in an Avanti J-31 Beckman refrigerated centrifuge to obtain the plasma. Aliquots of plasma were taken for the various biochemical assays: 100 μl for the FFT and 20 μl for total L-tryptophan (difference between total and FFT was considered to be the albumin-bound fraction), 200 μl for NAAs, 25 μl for albumin, 50 μl for free fatty acids (FFAs), 20 μl for glucose, and 50 μl for HbA1c (A1C).

**Biochemical assays**

For plasma L-tryptophan, ultrafiltered plasma fractions were obtained (Nanosep 30K with Omega; Pall, Ann Arbor, MI), in which the FFT was recovered. The high performance liquid chromatography-fluorescence method of Peat et al. (19) was used to quantify FFT and total L-tryptophan. Plasma albumin was quantified by the method of Doumas et al. (20). Plasma glucose was determined by the method of glucose oxidase (21), A1C and (22) NAAs by high-performance liquid chromatography (23), and FFAs were quantified by the Wako NEFA C test kit that utilizes an in vitro enzymatic colorimetric method.

**Recording of N1/P2 components of the auditory-evoked potentials**

Recordings took place in an electrically shielded and sound-attenuated room adjacent to the recording apparatus (Viking 4; Nicolet). Children were seated in a slightly reclined chair with a headrest. Evoked responses were recorded with two channels referred to vertex (Cz). AgCl electrodes were used (electroencephalographic disk electrode NE-101, 10 mm diameter). A total of 200 tones (1 kHz, 100 ms duration with 10 rise and fall times, and interstimulus interval between 1,000 and 1,500 ms) with four intensities (40, 60, 90, and 103 dB) to assess the intensity dependence were used. Intensities were each presented separately binaurally in a sequential form through headphones. Data were collected with a sampling rate of 1,000 Hz and an analogous bandpass filter (0.1–150.0 Hz). Pre-stimulus (200 ms) and poststimulus periods (500 ms) were evaluated for 200 sweeps of every intensity. For artifact suppression, all trials were automatically excluded from averaging if the voltage exceeded 50 μV in any of the two channels at any time point of the averaging period. The X-Y graphs of the auditory-evoked potentials were examined, and prominent peaks were identified and measured using specific software (Viking 4; Nicolet). The plots shown (Fig. 1) are illustrative examples of IDAEP's obtained at sequential stimulations of 40, 60, 90, and 103 dB sound pressure level in a control child (Fig. 1A) and a patient with type 1 diabetes (Fig. 1B). Latencies in milliseconds and amplitudes in microvolts were also calculated. The amplitude of the N1/P2 component of the auditory evoked potentials was considered as the sum in microvolts between the crests of the waves N1 and P2.

**Statistical methods**

Differences among mean value sets were analyzed for significance by Mann-Whitney U, Friedman ANOVA, and Levine tests with a level significance of P < 0.05. Peak-to-peak amplitude of the N1/P2 component was measured at 40, 60, 90, and 103 dB stimulus intensity, and the amplitude/stimulus intensity function (ASF) slope was calculated for each group of children (generalized linear model through ANOVA for repeated measures).

**RESULTS** — The glycemia, A1C, FFAs, and NAAs in plasma were significantly elevated in type 1 diabetic children (P < 0.01). Plasma albumin concentration was similar in both groups (type 1 diabetic and control subjects) (Table 1).

In accord with previous observations (9), children with type 1 diabetes showed a significant decrease of FFT, bound to albumin and total L-tryptophan, as compared with normal children (P < 0.01). The FFT-to-total L-tryptophan and FFT-to-NAAs ratios were significantly lower in the diabetic group (P < 0.01) (Table 2).

The latencies (milliseconds) of N1 and P2 recorded on Cz showed a tendency to increase with the intensity of the stimulus, from 68.6 ± 2.46 to 128.8 ± 9.47 (N1) and 102.4 ± 5.50 to 197.1 ± 12.46 (P2) in control subjects and from 80.8 ± 4.84 to 156.0 ± 5.49 (N1) and 140.5 ± 6.37 to 242.8 ± 6.38 (P2) in type 1 diabetic subjects (P < 0.001). The latencies of these components in the dia-
Betic children were higher in relation to control subjects ($P < 0.001$) (Friedman ANOVA test).

One of the main clinical parameters evaluated in these children was the IDAEP's N1/P2 components. It is important to note that the ASF slope in the patients showed a significant increase compared with the control subjects ($P < 0.05$). (Fig. 2) No significant correlation was found between the ASF slope of the IDAEP N1/P2 component and the FFT (results not shown).

**CONCLUSIONS** — The purpose of the present work was to evaluate the electric activity of the primary auditory cortex through the N1/P2 component of the IDAEP and the FFT and its ratio with NAAs as indicators of brain serotonin synthesis in children with type 1 diabetes. The present results of the biochemical analysis confirm metabolic changes observed in a former study in diabetic children (9); the FFT, and the FFT-to-total l-tryptophan and FFT-to-NAA ratios are significantly reduced. The decrease of FFT in plasma with a concomitant decrease of the FFT-to-NAA ratio suggest a decrease in the transport of the precursor amino acid to the brain related to a decrease in its availability at the BBB level that in turn may induce a decrease in the serotonin synthesis rate, similar to that observed in the brain of diabetic rats (10,11,24). The decrease in plasma FFT in type 1 diabetic children cannot be explained by the increase in FFAs that would tend to favor an increase because it is known that FFAs compete with l-tryptophan for binding to albumin (6). Rather, the decrease in plasma FFT can be explained by a deviation of l-tryptophan to other metabolic pathways such as those of kynurenic and nicotinic acid (25), which could mask a possible increase, with a final low FFT at the BBB level. On the other hand, in the diabetic state, there

**Table 1—Biochemical data in plasma of children with type 1 diabetes and control subjects**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>CV</th>
<th>Type 1 diabetic subjects</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>91.46 ± 6.52</td>
<td>0.071</td>
<td>126.2 ± 9.29*</td>
<td>0.007</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>5.43 ± 0.16</td>
<td>0.029</td>
<td>8.06 ± 0.53†</td>
<td>0.065</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.90 ± 0.35</td>
<td>0.071</td>
<td>4.30 ± 0.20</td>
<td>0.044</td>
</tr>
<tr>
<td>FFAs (mmol/ml)</td>
<td>0.52 ± 0.03</td>
<td>0.057</td>
<td>5.53 ± 0.16†</td>
<td>0.103</td>
</tr>
<tr>
<td>Valine (μmol/l)</td>
<td>80.65 ± 5.28</td>
<td>0.065</td>
<td>112.3 ± 12.07*</td>
<td>0.107</td>
</tr>
<tr>
<td>Isoleucine (μmol/l)</td>
<td>45.43 ± 2.70</td>
<td>0.059</td>
<td>78.60 ± 11.00*</td>
<td>0.139</td>
</tr>
<tr>
<td>Leucine (μmol/l)</td>
<td>37.43 ± 6.10</td>
<td>0.162</td>
<td>80.80 ± 10.71*</td>
<td>0.132</td>
</tr>
<tr>
<td>Phenylalanine (μmol/l)</td>
<td>33.75 ± 3.21</td>
<td>0.095</td>
<td>77.80 ± 10.50*</td>
<td>0.134</td>
</tr>
<tr>
<td>Tyrosine (μmol/l)</td>
<td>30.69 ± 6.37</td>
<td>0.207</td>
<td>50.01 ± 6.50*</td>
<td>0.129</td>
</tr>
</tbody>
</table>

Data are means ± SD of 23 determinations from 11 type 1 diabetic and 12 control children. All determinations were performed in duplicate samples. Differences were determined by Mann-Whitney U test. *$P < 0.01$, †$P < 0.001$. CV, coefficient of variation.
is a stimulation of liver tryptophan oxygenase activity that may activate L-tryptophan catabolism (25,26). The metabolic changes caused by the diabetic state on NAAs and FFT strongly suggest that brain serotonin synthesis might be reduced in this type of patient.

The IDAEP (N1/P2 component) has been proposed as an indicator of the activity of serotonergic neurons on the primary auditory cortex (12–15,27) where a low neuronal serotonergic activity leads to a high-intensity dependence with higher N1/P2 amplitudes, suggesting that there is a functional relationship between the actual brain serotonin activity with the N1/P2 changes. Therefore, alterations of the N1/P2 component of the IDAEP may reflect a cortical impaired activity associated with abnormalities of brain serotonergic neurotransmission. This is important because this component of the auditory cortex electrical activity (N1/P2) is the result of spatial and temporal integration of various neuronal processes. Electrical dipole source analyses have permitted the identification of two major components in each hemisphere (12,13): a tangential dipole source representing activation of the primary auditory cortex and a radial dipole generated by activity in structures of the secondary auditory cortex. Since N1/P2 is also induced in children by auditory stimulus and detected on the corresponding auditory projections on the scalp, it seems reasonable to accept that in children as in the adult, they reflect cortical integration of the auditory activity (16).

Alterations of the auditory activity expressed by changes in the IDAEP (N1/P2 component) have been assumed to be a consequence of a hypothetical central mechanism regulating the sensory sensitivity. According to this hypothesis, a reduction reflects a pronounced activity of the central mechanism protecting the organism from sensory overload, whereas an increase reflects the lack of such a protection (28). The measure of the ASF slope at various stimulus intensities supports the intensity dependence of the N1/P2 components. Following these concepts, the increase of the ASF slope observed in children with type 1 diabetes, in the present study, would indicate a diminution of this regulatory mechanism. Various authors (29,30) have suggested that such a mechanism acts at the level of the brainstem and is most likely represented by the serotonergic system. Serotonin has a homeostatic function in the central nervous system and acts to adjust and control gain factors and excitability levels of cortical neurons (3,31). The primary sensory cortices, in particular layer IV of the primary auditory cortex, contain a dense serotonergic innervation (32,33). Layer IV also receives most of the specific thalamic sensory input (34). Therefore, it has been proposed that serotonergic projections from the raphe nuclei in the brainstem modulate the initial signal processing in the sensory cortex. So, we propose, based on the present biochemical and electrophysiological results, that in children, the response of the auditory cortex to sound intensity stimulus may be also regulated by the current serotonergic activity, and in the case of children with type 1 diabetes a decreased serotonergic neurotransmission may provoke a different behavior of sensory cortices and the different auditory cortex response detected by auditory-evoked potentials as an ASF of the intensity dependent N1/P2 component. The same serotonergic changes that modify the acoustic evoked potential response from cortex may be involved in the thalamic corticofugal gating (35,36). Since there are abundant serotonin innervated GABAergic circuits in the sensory cortex, which act to inhibit the neuronal responses (5), it is possible that a reduction of the serotonergic modulation on the GABAergic neurons may enhance auditory cortical activity and its response to sound intensity and the amplitude of N1/P2. There is also an effect of type 1 diabetes on the latencies of N1/P2 that may be explained by a possible change in conductivity of the thalamocortical pathways (37,38).

In conclusion, these findings seem to have clinical relevance because brain serotonin is known to play an important role in the pathophysiology of various neuropsychiatric disorders that are commonly present in patients with type 1 diabetes like anxiety and depression (39). Therefore, we propose the use of the IDAEP (N1/P2 component) as a noninvasive electrophysiological indicator of changes in brain serotonin synthesis and activity in patients with type 1 diabetes.

Table 2—Plasma concentration of L-tryptophan in children with type 1 diabetes and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Type 1 diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CV</td>
<td>CV</td>
</tr>
<tr>
<td>FFT</td>
<td>0.035</td>
<td>0.012</td>
</tr>
<tr>
<td>Albumin bound</td>
<td>0.030</td>
<td>0.012</td>
</tr>
<tr>
<td>Total</td>
<td>0.023</td>
<td>0.022</td>
</tr>
<tr>
<td>FFT-to-total ratio</td>
<td>0.023</td>
<td>0.022</td>
</tr>
<tr>
<td>FFT-to-NAA ratio</td>
<td>0.023</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Data are in μmol/l and are means ± SD of 23 determinations from 11 type 1 diabetic and 12 control children. All determinations were performed in duplicate samples. Differences were determined by Mann-Whitney U test. *P < 0.001. CV, coefficient of variation.

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References


