

Clinical and Basic Studies on Diabetes and Brain Serotonin

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Abstract

Objective: The aim of this review was to assess the hypothesis that type 1 or 2 diabetes mellitus produced a decrease of brain serotonergic neurotransmission through measurement of plasma free fraction of L-tryptophan (FFT) and intensity-dependence auditory-evoked potentials (IDAEPs).

Methods: A diabetic mellitus (DM) model was produced through the administration of streptozotocin. After 7 days, the diabetic rats were divided into two groups. One group was treated with insulin and the other did not receive treatment. Their brainstems were removed to determine: L-tryptophan (L-Trp), 5-hydroxytryptamine (5-HT), and tryptophan-5-hydroxylase activity (TPH), besides its kinetics and activation through phosphorylating conditions. In addition, a series of cross-sectional studies were carried out in patients with diabetes type 1 and 2, with and without depression, in their plasma we determined FFT, and other amino acids unrelated to 5-HT metabolism; glucose and glycosylated hemoglobin. The IDAEP's N1/P2 components were also determined.

Results: A decrease in the synthesis of brain serotonin during the diabetic state was confirmed, due to changes in the kinetics and phosphorylation capacity of TPH and a decreased expression of the THP molecule, which did not return to normal levels with insulin. Also, all diabetic patients showed a significant decrease of FFT in the plasma. The diabetic patients presented an increase in the slope of the amplitude/stimulus intensity function (ASF slope) of the N1/P2 component. It is interesting to mention that the ASF slope was steeper in the diabetic patients with depression in relation to patients diabetic but without depression.

Conclusions: The decrease of the FFT in plasma and the increase the N1/P2 component's amplitude may reflect a functional relationship between the brain serotonergic tone and the diabetic metabolic changes. The increase of the ASF slope in patients with type 1 diabetes suggest that the response of the auditory cortex to sound intensity stimulus may be regulated by the brain serotonergic activity 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 patients with diabetes type 1 and 2. These changes may be related to the psycho-emotional manifestations observed in diabetic children like anxiety and depression.

Keywords: Type 1 or 2 diabetes; Brain serotonin; L-tryptophan; Tryptophan-5-hydroxylase; Auditory-evoked potentials (N1/P2 component)

Introduction

The study of peripheral markers of the brain serotonergic system in diabetic patients represents an opportunity to evaluate how the metabolic changes due to diabetes Mellitus (DM) 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 [1-3]. The specific neurotransmitter is serotonin (5-hydroxytryptamine, 5-HT), also acting as a neuromodulator which activates a large family of G protein-and ion-coupled, metabotropic receptors [3-5]. 5-HT is synthesized from L-tryptophan (L-Trp). L-Trp is an essential amino acid and the precursor for the biosynthesis of brain serotonin. There are two known fractions of plasma L-Trp: one bound to albumin, and another free [6]. The free fraction traverses the blood-brain barrier (BBB) and is taken up by serotonergic neurons to activate the synthesis of the neurotransmitter, 5-HT [7-9]. There are several mechanisms proposed for the regulation

of the amount of plasma L-Trp passing to the brain. One is a specific transport system [10] and another depends on its not binding to albumin [6]. These two mechanisms may compete at the BBB level [11] it is possible that the higher affinity of the carrier system at the brain capillary wall would strip L-Trp from albumin and increase its transport to the brain. A third regulator would be through the competition of the neutral amino acids (Phenylalanine, Valine, Leucine, Tyrosine, Isoleucine, NAA), which seem to share the carrier that transports L-Trp to the brain [12-15]. In the brain, L-Trp is hydroxylated in serotonergic neurons by the action of the enzyme tryptophan hydroxylase (EC 1.14.16.4, TPH) [16-19]. 5-Hydroxytryptophan is then decarboxylated to 5-HT [20,21].

We have reported a specific change in the serotonergic system during the diabetic state, which consists of a decrease in the biosynthesis of serotonin due to a reduction in free fractions of L-tryptophan (FFT) in plasma and brain, together with a chronic inhibition of the enzyme tryptophan-5-hydroxylase activity [22,23]. The chronic decrease of L-Trp in the brain and in the TPH activity in animals with DM, as well as the

kinetic changes of TPH that consist of an increase of K_m for L-Trp and a decrease of V_{max} and a lower phosphorylation activity, where the inositol 1,4,5 triphosphate (IP₃), diacylglycerol, calcium/calmodulin-dependent protein kinase II, and cyclic adenosine monophosphate (cAMP), seem to be the mechanisms involved in the low activation of this metabolic cerebral pathway caused by DM [24].

However, when animals with DM were submitted to treatment with insulin they show a complete recovery in body weight and the brain L-Trp also returns to normal. Despite these physical and biochemical recoveries, the TPH activity remained impaired. This seems to indicate that the changes suffered by the enzyme system could be due to a different cause other than substrate changes, for instance, the fact that TPH activity remains inhibited after insulin treatment supports the possibility of a different pathophysiologic mechanism [23]. Recently we have reported that the expression of both isoforms of the TPH, in non-insulin treated rats, was significantly decreased in comparison to control groups. However, it is important to mention, that while the diabetic rats treated with insulin showed a return to normal L-Trp concentration, the expression of both isoforms remained significantly decreased during the evolution of the diabetic state [25]. These findings tend to confirm that in diabetic rats treated with insulin, the mechanism of inhibition of the biosynthesis of brain serotonin may not be only due to a change related to the concentration of L-Trp. There is also a possibility that the outcome of all the metabolic changes involved in DM may be, among other, an alteration in the expression of the enzyme protein itself through mechanisms independent of specific encoding genes, similar to what has been shown during social stress [26-28], with a negative impact on the biosynthesis and functionality of this important neurotransmitter.

On the other hand, it is interesting and relevant for the present review that decreased 5-HT availability in the brain induces also an important functional brain alteration; it increases neuronal cortical activity in the auditory cortex (A1). This disturbance in cortical activity has been detected as a change in the N1/P2 component's amplitude of the intensity dependent induced auditory potentials (IDAEP). An opposite effect is also observed when 5-HT neuronal activity increases on the auditory cortex [29-34], as we have observed in early malnourished rats and infants [35]. Indeed, these same conditions seem also to occur in human babies what suffered intrauterine growth restriction [36]. The IDAEP's N1/P2 component recorded from the scalp consists mainly of two overlapping subcomponents produced by two brain structures: there is converging evidence from intracranial recordings that the superior temporal plane and the lateral gyri are the main generators of these subcomponents. The N1 component of the individual dipole source is measured as the negative peak within 60 to 120 ms, and the P2 component is measured as the positive peak within 110 to 210 ms [29-31,33,35,37]. So, it is accepted that these components are representative of auditory cortex integrative functions [38]. Therefore, here we have proposed the hypothesis that in humans with type 1 or 2 diabetes, the free fraction of L-Trp in the plasma and the IDAEP's N1/P2 components may be also altered, reflecting brain changes in serotonergic neurotransmission. Quantification of these parameters evaluated in humans, can be useful as parameters of brain 5-HT synthesis.

Methods

To establish further support for this hypothesis, comparative cross-sectional studies were carried in various groups of type 1 and 2 diabetics with and without depression [39-41]. During the diabetic state were determined in their plasma free and bound L-Trp, and other amino acids unrelated to 5-HT metabolism; albumin, free fatty acids, glucose and glycosylated hemoglobin and the IDAEP's N1/P2 components. None of the diabetic patients was in remission. A brief description of these studies follows: The first study was formed by 34 children, 6.83-10.49

years of age, selected from the Endocrinology Service of the Pediatric Hospital, XXI Century, National Medical Center, Mexican Institute of Social Security, Mexico City, Mexico [39]. Two groups were formed: The first group included 22 children with type 1 diabetes, according to the National Diabetes Group criteria, with a body mass index (BMI) normal for their age and without other underlying diseases. The second group was made up of 12 normal children within the same age range who served as control subjects. All children were fed a normal diet of 55 kcal/kg/day (protein 30%, carbohydrates 55%, lipids 15%). Additionally, patients with diabetes were treated with a mixture of fast and intermediate-action insulin, 0.8-1U/kg/day. Three ml of blood were collected by venopuncture in borosilicate tubes containing 450 μ L of ACD solution, which consisted of 3.6 mg sodium citrate, 9.9 mg citric acid, 11 mg dextrose, buffered with 50 mmol Tris acetate, pH 7.40 between 07:30 and 08:30 AM and 12 hours after the last feeding. The tubes containing the blood samples were cooled immediately (0-4°C) on ice and centrifuged at 500 g in a refrigerated centrifuge to obtain the plasma sample. Aliquots were taken for the following biochemical assays: 100 μ L for the FFT and 20 μ L for total L-Trp (difference between total and FFT, was considered to be the albumin bound fraction), 200 μ L for neutral amino acids (NAA), 25 μ L for albumin, 50 μ L for free fatty acids, 20 μ L for glucose and 50 μ L for glycated hemoglobin.

A second study was planned in 23 children selected from the Service of Endocrinology. Two groups were formed [40]. The first group included 11 children of both sexes with type 1 diabetes, aged 10.9 ± 0.39 (mean \pm 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. The second group was made up of 12 non-diabetic 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. Children with type 1 diabetes were managed with a combination of fast- and intermediate-action insulin, 0.8-1.0 U/kg/day. Two ml of blood were collected and processed for assays as described above.

Results

Patients with type 1 diabetes had an evolution of 4.4 ± 2.7 years, when the study was performed. There were no differences in anthropometric data when compared with control children (Table 1). Glycemia and glycosylated hemoglobin were significantly elevated in patients with diabetes. Furthermore, these children had an increase in free fatty acids (Table 2) and NAA in plasma, when compared with normal children (Table 3). Note that the plasma albumin of both groups of children was similar (Table 2).

In the same way that the previous diabetic group, there were no differences in anthropometric data when compared with control children. Regarding glycemia, glycated hemoglobin, free fatty acids, and neutral amino acids in plasma, they were significantly elevated in these type 1 diabetic children.

Data	T1DM	C
Age (years)	8.66 ± 1.83	8.25 ± 1.05
Sex		
Males	10	8
Females	12	4
Body weight (kg)	30.82 ± 6.75	27.11 ± 5.46
Length (m)	1.30 ± 0.10	1.28 ± 0.03
Body mass index	17.35 ± 2.00	17.13 ± 2.18
Time of evolution (years)	4.41 ± 2.70	-

Table 1: Clinical data of schoolchildren with Type 1 Diabetes mellitus (T1DM) and controls (C)

Each point represents the mean value \pm SD. Differences were determined by Student *t* test.

Data	T1DM	C	p
Glucose (mg/dL)	209.14 ± 10.66 ^u	90.55 ± 15.61	0.001
Glycosylated hemoglobin (%)	9.23 ± 3.37*	4.65 ± 0.54	0.01
Albumin (g/dL)	4.63 ± 0.10	5.00 ± 0.25	
Free fatty acids (mmol/mL)	1.75 ± 0.16*	0.55 ± 0.02	0.01

Table 2: Biochemical data in plasma of schoolchildren with Type 1 diabetes mellitus (T1DM) and controls (C)

^u*Each point represents the mean value ± SD of 34 determinations from 22 T1DM patients and 12 controls. All determinations were performed in duplicate. Differences were determined by Student *t*-test.

AMINO ACIDS	T1DM	C	p
Valine	112.3 ± 12.07*	80.65 ± 5.48	0.01
Isoleucine	78.86 ± 11.08*	46.43 ± 4.70	0.01
Leucine	78.86 ± 11.08*	46.43 ± 4.70	0.01
Phenylalanine	49.86 ± 6.63*	33.75 ± 3.21	0.01
Tyrosine	66.76 ± 12.03*	30.69 ± 6.37	0.01

Table 3: Plasma concentration of neutral amino acids in schoolchildren with Type 1 diabetes mellitus (T1DM) and controls (C)

*Each point represents the mean value ± SD (μmol) of 34 determinations from 22 T1DM patients and 12 controls. All determinations were performed in duplicate sample. Differences were determined by Student *t* test.

In the course of other studies in diabetic patients with or without depression [41], we observed that the FFT in plasma and its ratio to neutral amino acids were also significantly reduced, suggesting a decrease in the transport of the precursor amino acid to the brain and in the serotonin synthesis rate, similar to that observed in diabetic animals [22-25]. This finding may be of relevance in the pathophysiology and in the clinical picture, which seems to be related to an alteration of 5-HT metabolism and neurotransmission in the brain and to the neuropsychiatric disorders in diabetic patients. Thus, we have proposed that the free fraction of L-tryptophan and its ratio to neutral amino acids in plasma may be clinically useful as indicators of brain serotonergic activity in these patients.

Besides, in the IDAEP represented by the N1/P2 component, the slope calculated as a function of the amplitude in mV, of component N1/P2 with the intensity of the stimulus, is known as the ASF slope. In patients with diabetes, this function showed a significant increase compared with the controls ($P < 0.05$) (Figures 1 and 2). It is interesting to mention that in another study, this ASF slope was steeper in diabetic patients with depression in relation to the diabetic patients without depression. In patients with only depression ASF was similar to that of diabetic patients, but less than the ASF in diabetic patients with depression [41].

We have also studied other possible functional alterations in a group of women with type 2 diabetes in order to get further information on their serotonergic metabolism and related brain function [42]. The diabetic patients were overweight and a reduction in FFA and NAA was also confirmed in plasma together with a decreased free, bound to albumin and total L-Trp [42].

Discussion

From these results we concluded that the decrease of the FFT in plasma and the increase in the N1/P2 component amplitude do reflect metabolic and functional disturbances secondary to the diabetic state, and that there is a relationship of ASF and the actual modified serotonergic activity in the auditory cortex (A1), suggesting that the response of the A1 to sound intensity stimuli may be regulated by the serotonergic tone whose decrease

due to the metabolic changes described, may provoke a different behavior of the sensory cortex, including the auditory cortex [30-36]. Therefore the IDAEP-N1/P2 component may be an electrophysiological indicator of brain changes of the serotonergic neurotransmission in children with type 1 diabetes. The altered responses of the sensory cortex to environmental stimuli might be related to the psychoemotional manifestations observed in diabetic children, such as anxiety and depression [41,43-46]. It is interesting and relevant for this study that decreased 5-HT availability in the brain does increase neuronal cortical activity in A1 and as reported in the auditory cortex too, that could be detected as an increase in the N1/P2 component's amplitude of the IDAEP [34,40,41]. The opposite effect is also observed when 5-HT neuronal activity is elevated in A1, as we have observed in early malnourished rats [35]. This same condition seems also occur in human babies with intrauterine growth restriction [36].

Results of biochemical analyses confirm the metabolic changes observed in a former study in diabetic children [39]: The FFT and the FFT-to-total L-Trp and the FFT-to-NAA ratios were significantly reduced. The decrease of FFT in plasma with the concomitant decrease of FFT-to-NAA ratio suggest a decrease in the transport of the precursor amino acid to the brain that leads to a reduction of its availability at the 5-HT synthesis path, similar to that observed in the brain of diabetic rats [22-25]. The low plasma FFT in type 1 diabetic children cannot be explained by the increase in FFA (free fatty acids) that normally would tend to favor an increase [41], because it is known that FFA compete with L-Trp for binding to plasma albumin [6]. Rather, the decrease in plasma FFT may be explained by a deviation of L-Trp to other metabolic pathways such as those of kynurenic and nicotinic acid [47,48], which could mask a possible increase, resulting in a final low FFT at the BBB level. On the other hand, in the diabetic state, there is stimulation of liver tryptophan oxygenase activity that may activate L-Trp catabolism [47,48]. The metabolic changes caused by the diabetic state in the plasma.

Alterations of the auditory cortex 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 [49]. The measure of the ASF slope at various sound stimuli intensities supports the intensity-dependence of the N1/P2 component. Following these concepts, the increase of the ASF slope observed in diabetic patients in our studies [40,41], would indicate a deficiency of this regulatory mechanism. Interestingly various authors [50-52] have suggested that such a mechanism acts at the level of the brainstem and is most likely regulated by the serotonergic system [3]. Serotonin has a homeostatic function in the central nervous system and acts to adjust and control gain factors and excitability levels of cortical neurons [3,52]. The primary sensory cortices, in particular layer IV of the primary auditory cortex, contain a dense serotonergic innervation [53,54]. Layer IV also receives most of the specific thalamic sensory input [55]. Therefore, it has been proposed that serotonergic projections from the raphe nuclei in the brainstem do modulate the initial signal processing in the sensory cortex. So, we propose, based on the reported biochemical and electrophysiological results, that in diabetic patients, the response of the auditory cortex to different 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, as well, a different behavior of the sensory cortices and the different auditory cortex response detected by IDAEP, as an altered 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 [56,57]. Since there are abundant serotonin innervated GABAergic circuits in the sensory cortex, which act to inhibit

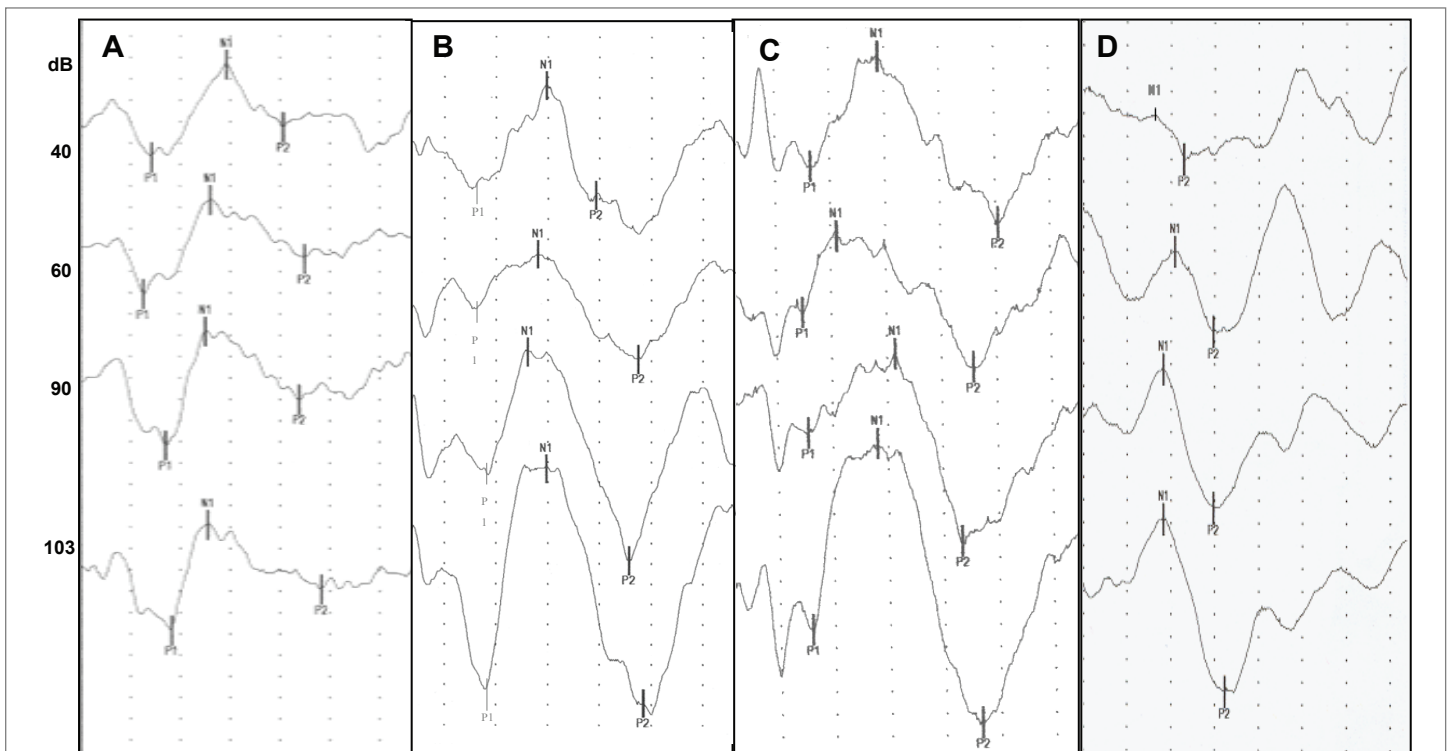


Figure 1: Illustrative examples of cortical auditory-evoked potentials (200 averaged responses) obtained at separate stimulations of 40, 60, 90 and 103 dB sound pressure level. (A) Control adolescent. (B) Patient with type 1 diabetes mellitus. (Notice the significant increase in the amplitude of the N1-P2 component) (C) Patient with type 1 diabetes mellitus and depression. (D) Patient with depression without diabetes. Peak-to-peak amplitude of the N1/P2 component was measured at each stimulus intensity. Reproducibility tested by Levene and coefficient of variation tests.

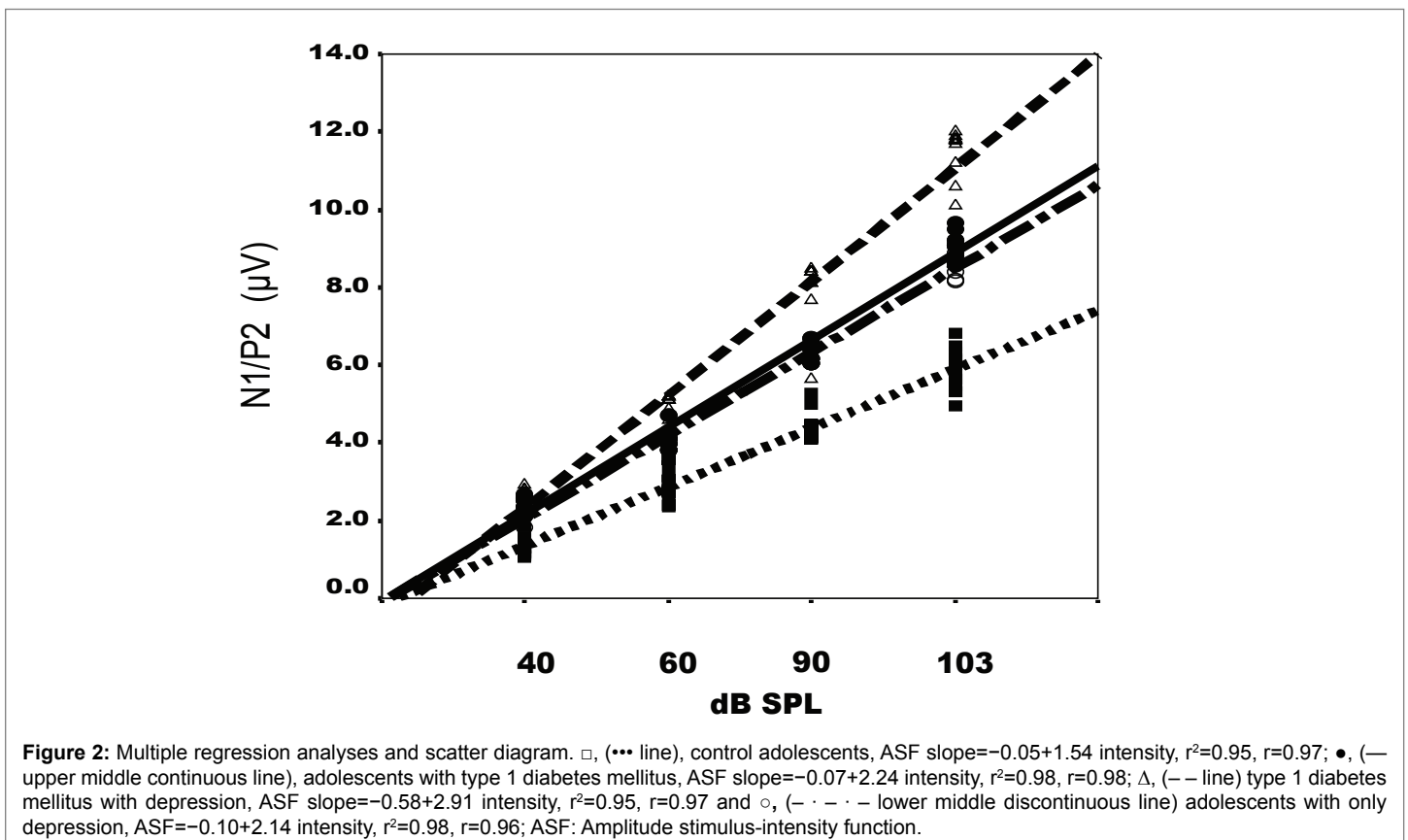


Figure 2: Multiple regression analyses and scatter diagram. \square , (— line), control adolescents, ASF slope= $-0.05+1.54$ intensity, $r^2=0.95$, $r=0.97$; \bullet , (— upper middle continuous line), adolescents with type 1 diabetes mellitus, ASF slope= $-0.07+2.24$ intensity, $r^2=0.98$, $r=0.98$; Δ , (--- line) type 1 diabetes mellitus with depression, ASF slope= $-0.58+2.91$ intensity, $r^2=0.95$, $r=0.97$ and \circ , (- · - · - lower middle discontinuous line) adolescents with only depression, ASF= $-0.10+2.14$ intensity, $r^2=0.98$, $r=0.96$; ASF: Amplitude stimulus-intensity function.

the neuronal responses, it is possible that a reduction of the serotonergic modulation on the GABAergic neurons [5] may enhance auditory cortical in activity and its response to sound intensity and the amplitude of N1/P2.

Conclusions

The findings here reviewed 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 [41,43-46]. Therefore, we propose the clinical use of the IDAEP (N1/P2 component) as a noninvasive electrophysiological indicator of changes in brain serotonin activity in patients with type 1 and 2 diabetes.

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Conflict of Interest

The authors declare no conflicts of interest.

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