



Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 4(10) pp. 441-448, October, 2015
Available online <http://garj.org/garjmms>
Copyright © 2015 Global Advanced Research Journals

Full Length Research Paper

Brain Serotonergic Disturbances Caused by Diabetes Mellitus are not Reversed by Insulin Treatment

Gabriel Manjarrez-Gutiérrez^{1,2*}, Teresa Neri-Gómez¹, Rocío Herrera-Márquez¹, José Antonio Mondragón-Herrera¹, Alfonso Boyzo-Montes de Oca³ and Jorge Hernandez-Rodríguez³

¹Laboratory of Molecular Pathology, Cardiology Hospital, National Medical Center (CMN-SXXI), Mexican Institute of Social Security (IMSS), Mexico City, Mexico

²Medical Research Unit in Neurological Diseases, Specialties Hospital, CMN-SXXI, IMSS, México, DF, México

³Laboratory of Neurontogeny, Department of Physiology, Biophysics and Neurosciences, Center of Research and Advanced Studies (CINVESTAV) IPN, Mexico City, Mexico.

Accepted 05 October 2015

The aim of this study was to determine whether changes in the kinetics and the capacity of phosphorylation of the tryptophan-5-hydroxylase (TPH) in diabetic rats are normalized with insulin, or if there are changes in expressions of TPH1 or TPH2 as mechanisms that explain the chronic inhibition of the biosynthesis of brain 5-hydroxytryptamine (5-HT). A diabetic mellitus (DM) model was produced through the administration of streptozotocin and controls (STZ). After 7 days, the diabetic rats were divided into two subgroups. One group was treated with insulin and the other did not receive treatment. After 7, 14 and 21 days, the brainstem was removed to determine: L-tryptophan (L-Trp), 5-HT, TPH activity, by observing the kinetics and activation through phosphorylating conditions. Besides, the expressions of TPH1, TPH2 and TPH-phosphorylated-S19 (TPH2-^PS-19) were assessed by Western blot. The diabetic rats had a decrease of L-Trp, 5-HT and TPH activity; a decrease of the V_{max} and an increase in K_m . They also showed less activation of the TPH by phosphorylation and a reduced expression of TPH1, TPH2 and TPH2-^PS-19. Interestingly enough, all these changes are not normalized in the diabetic rats subgroup treated with insulin. 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 TPH1, TPH2 and TPH2-^PS-19, which did not return to normal levels with insulin. These findings support the fact that chronic inhibition of 5-HT biosynthesis resistant to insulin is due to the decrease of TPH's expression and activity as a consequence of metabolic changes during the diabetic state.

Keywords: Brain, Diabetes mellitus, Insulin, Serotonin, Tryptophan-5-hydroxylase

INTRODUCTION

Tryptophan-5-hydroxylase (TPH) is the rate-limiting

enzyme of the cerebral biosynthesis of 5-hydroxytryptamine (5-HT, serotonin) (Grahame-Smith, 1964; Jequier et al., 1969; Neckers et al., 1971). It is a tetramer of identical subunits with a molecular weight ~240kDa. There are two isoforms of the enzyme, TPH1 (located in peripheral tissues) and TPH2 (located

*Corresponding Author E-mail: willisga@prodigy.net.mx, gmanjarrezg@gmail.com; Phone: (+52) (55) 56276900, Ext. 22156; Fax: (+52) (55) 55780240;

in the central nervous system (CNS) (Veenstra and Cook, 2003; Walther, 2003; Walther and Bader, 2003; Patel et al., 2004). These enzymes require Fe^{2+} in the presence of oxygen to be activated (Hufton et al. 1995; Fitzpatrick, 2000; Walther, 2003). TPH has three functional regions: an amino terminal regulatory domain, a catalytic domain and a terminal carboxyl interface. The last region is responsible for the tetrameric structure and catalytic activity due to the presence of an α helix region (Moran et al., 1998; Fitzpatrick, 2000). In this molecular region, the following areas are found: Fe^{2+} junction (cofactor), tetrahydrobiopterin (BH_4), and the substrate L-tryptophan (L-Trp) (Moran et al., 1998; Fitzpatrick, 2000). TPH catalyzes the insertion of molecular oxygen at position five from the L-Trp, whereas the other oxygen atom is reduced to water. This reaction requires BH_4 (Manjarrez et al., 2010), an electron donor, to reduce oxygen to form hydroxyls. Thus, L-Trp is converted into 5-hydroxytryptophan, which immediately is decarboxylated by the amino acids to produce 5-HT (Neckers et al., 1971; Kaufman, 1985; Boadle-Biber, 1993).

Recent studies show 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 (Manjarrez et al., 1999; Manjarrez et al., 2000). The chronic decrease of L-Trp in the brain and TPH activity in animals with DM, as well as the kinetic changes of TPH, consist in an increase of K_m for L-Trp and a decrease of the V_{max} , and lower phosphorylation activity, where the inositol 1,4,5 triphosphate (IP_3), 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 (Herrera et al., 2005). These same conditions seem to occur also in patients with type 1 and 2 diabetes, with or without clinical depression and in patients with Metabolic Syndrome, since their FFT is also significantly diminished (Herrera et al., 2003; Manjarrez et al., 2006; Manjarrez et al., 2007; Manjarrez et al., 2009; Herrera et al., 2011).

However, when animals with DM were submitted to treatment with insulin they show a complete recovery in body weight and brain L-Trp also returns to normal (Manjarrez et al., 2000). Despite the physical and biochemical recovery, 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 physiopathologic mechanism. Because of these results, this study was proposed to obtain further information on the biochemical factors such as kinetic alterations and phosphorylation capacity and see if they remain altered

or are normalized under insulin treatment and assess if there is a change in the expression of TPH's isoforms. Our objective is to find out whether this is the main mechanism that may explain the chronic inhibition brain serotonin biosynthesis during the diabetic state.

MATERIALS AND METHODS

Sixty male Wistar rats, with an average body weight of 250 ± 10 g were used. For the adaptation period they were kept for two weeks under the following environmental conditions: Temperature $22 \pm 2^\circ\text{C}$, light and darkness cycles of 12 h; minimal noise and handling. During this period, the animals were fed rodent diet lab pellets (Nutritional International, Brent Wood, MO, USA) and water *ad libitum*. After this adaptation period the rats were divided in the following groups: A diabetic group (D) of 40 rats were administered 65 mg/Kg weight i.p. of streptozotocin (STZ), (Sigma Chemical Co. St Louis, USA), diluted in citrate buffer 0.1 M, pH 4.5; and a control group (C) of 20 rats that received only the vehicle solution. After seven days of STZ administration, 30 rats from the D group were treated with intermediate action insulin (10U/Kg b.w). These rats in particular formed the insulin treated diabetic group (ITD). All animals had food and water *ad libitum*.

After 7 days of the administration of STZ, animals from each group was sacrificed and their brainstems were dissected out on an ice-cold plate, weighed and homogenized in Tris-HCL buffer 50 mM, pH 7.40, plus 1.0 mM EGTA and 1mM 1,4-dithioerythritol. In these tissue samples, L-Trp, 5-HT and TPH activity were determined. The kinetic analysis of TPH activity was also performed, using a 29,000 g supernatant (see biochemical assays).

Other samples of brain stems were similarly obtained from each group on days 14 and 21 after STZ administration and processed as previously described. TPH activity was assessed in the presence of 0.5 mM ATP, 5mM MgCl_2 and 0.1 mM CaCl_2 . Additionally, other phosphorylating system was also explored with N^6, O^2 -dibutyl adenosine-3':5'-cyclic monophosphate (dibutyl cAMP). The results of these trials will be referred to as phosphorylated diabetic and control groups. Expressions of TPH1, TPH2 and TPH2- $\text{P}^{\text{S-19}}$ were also determined by Western blot, with monoclonal antibodies specific for each of the isoforms. Plasma glucose was measured by a system called One Touch (Life Scan. Inc, Milpitas, CA, USA).

In order to decrease any possible variations in the circadian rhythm, all experimental manipulation and dissection procedures were always made between 09.00 and 11.00 h. The rats were obtained from the animal facility of CINVESTAV-IPN (Mexico City). All animal experimental procedures were carried out in accordance with the Guidelines for the Care and Use of Experimental

Animals published by the Ministry of Health (Mexico). The protocol was approved by the Research Committee of the Cardiology Hospital, CMN-SXXI. General management of experimental animals was conducted in the Production Unit and Laboratory of Animal Experimentation (UPEAL, CINVESTAV-IPN) under health standards and ethics of the Internal Committee for the Care and Use of Laboratory Animals (IACUC), conforming with the Official Mexican Norm (NOM-062-ZOO-1999) (August 22, 2001).

Biochemical assays

Concentrations of 5-HT and L-Trp in the brainstem were determined by high-performance liquid chromatography (HPLC) according to the method of Peat and Gibb (1983). Briefly, all tissues were homogenized with a solution of HClO_4 0.1 M plus 4 mM sodium metabisulfite. The homogenized solution was centrifuged at 15,000g for 15 min at 4°C. Subsequently, 20 μl of the supernatant was injected into the HPLC (Waters Corporation, Milford, MA, USA) using a C_{18} reversed-phase symmetry column (5 μm particle size, 3.9 x 150 mm in length). A binary system of a solution of monobasic potassium phosphate (2 mM, pH 3.40) plus heptanosulfonic acid (1 g/L of solution) was used and a mixture of methanol/water at a ratio of 3:2v/v at a 1 mL/min rate. Determinations of 5-HT and L-Trp were performed using a fluorometric detector (Model 474, Waters Corporation) with a 290 nm excitation and 330 nm emission. The response was obtained by an analogous system (Empower 2, Waters Corporation) and concentrations of 5-HT and L-Trp were considered as maximum height of the signal according to a standard curve of known quantities of 5-HT and L-Trp (0.6, 25 and 100 ng/L). Results were expressed as μmol of serotonin/mg of tissue and μmol of L-Trp/mg of tissue.

TPH activity in the brainstem was assessed by determining the amount of 5-hydroxytryptophan formed by HPLC with a fluorometric detector (Model 474, Waters Corporation) (Johansen et al. 1995; 1996). Briefly, this method involved incubating 300 μg of enzyme protein in the presence of a buffer solution of Tris-HCl 50 mM, pH 7.4, 1.0 mM EGTA, 15 μg catalase, and 200 μM 2-amino-4-hydroxy-1-methyl tetrahydrobiopterin. Also the TPH activity stimulated by phosphorylation was determined in the presence of 0.5 mM ATP, 5 mM MgCl_2 and 0.1 mM CaCl_2 and 2.5 mM dibutyryl cAMP. In addition, the kinetic of the enzyme was measured using L-Trp concentrations ranging from 0.025 to 0.4 mM. The reactions were incubated at 37°C for 10 min and then stopped with the addition of HClO_4 6 M plus 5 mM EDTA and 0.1% ascorbic acid. Twenty μL of reagent was injected into the HPLC. C_{18} symmetry column (5- μm particle size, 3.9 x 150 mm) was used. The mobile phase

was prepared with 40 mM sodium acetate, pH 3.30, and acetonitrile at a ratio of 95:5, respectively, and run at 1 mL/min. Lengths of excitation and emission used for detection of 5-hydroxytryptophan were 280 nm and 340 nm, respectively.

Immunotransference of TPH by Western blot

Brainstems were homogenized for 30 seconds at 4°C in a solution of 50 mM Tris-HCl, pH 7.4, plus protease inhibitors (Protease Inhibitor Cocktail, Sigma-Aldrich Quimica SA de CV, Mexico). The samples were then centrifuged at 29,000 g for 15 min at 4°C. Protein concentration was quantified by the Bradford method. Then, 30 μg of protein were placed in each of the 1-mm-thick channels of an SDS-polyacrylamide gel at 12%. Electrophoretic run was performed at 100 V for two hours. For protein electrotransference, the gel was mounted on nitrocellulose membranes and the run was performed at 10 V, 1.30 mA for, 90 minutes. Nitrocellulose membranes with the transferred proteins were placed in a blocking solution (Millipore Chemiluminescent Blocker) at 50% for 30 minutes. The membranes were incubated with monoclonal primary antibody specific for TPH1 (Gene Tex, International Corporation, USA), TPH2 (Merck-Millipore, USA) and TPH2-^PS-19 (Abcam Cambridge MA, USA) at a dilution of 1:500 in the same blocking solution. On the following day, membranes were incubated with secondary anti-mouse antibody (Chemicon, USA) at a dilution of 1:5000 in blocking solution. Membranes were revealed with chemiluminescence (Millipore, corporation, Billerica, U.S.A) and exposed to a film (Kodak). The internal control used was glyceraldehyde-3-phosphate dehydrogenase (GADPH). The bands obtained were analyzed and quantified by densitometry.

Statistical method

In order to compare the results of serotonergic activity (L-Trp and 5-HT) and TPH activity, as well as, the relative optical densities of bands the TPH1, TPH2 and TPH2-^PS-19 were obtained by Western blot in the brainstem. In each of the groups, averages and standard deviations were first calculated. Afterwards, the groups were compared by ANOVA and Mann-Whitney U test due to non-normally distributed variables; $p < 0.05$ was accepted as statistically significant. Also, the kinetic analysis of TPH was done using a computer program Graph Pad Prism 5. The differences between mean values of the K_m and V_{max} of the groups were analyzed by ANOVA and Student's *t*-test with a level of significance of $p < 0.05$.

Table 1. Plasma glucose concentration

| Days post- administration of STZ | C | D | ITD |
|----------------------------------|-------------|---------------|---------------|
| 7 | 5.50 ± 0.11 | 18.10 ± 0.33* | 18.10 ± 0.33* |
| 14 | 5.66 ± 0.23 | 33.00 ± 0.50* | 5.95 ± 0.20 |
| 21 | 5.50 ± 0.36 | 34.32 ± 1.40* | 5.11 ± 0.62 |

Each value represents the mean ± SD (mmol/L) from twenty rats in each group. C, controls; D, diabetics and ITD, insulin treated diabetics. All determinations were performed in duplicate samples. The differences between groups was obtained by ANOVA (Treatment: Df = 19, SS = 65.46, MS = 18.465, F = 180.6. Time: Df = 19, SS = 0.88, MS = 19.78, F = 0.621) and Student's *t*-test. **p* < 0.001.

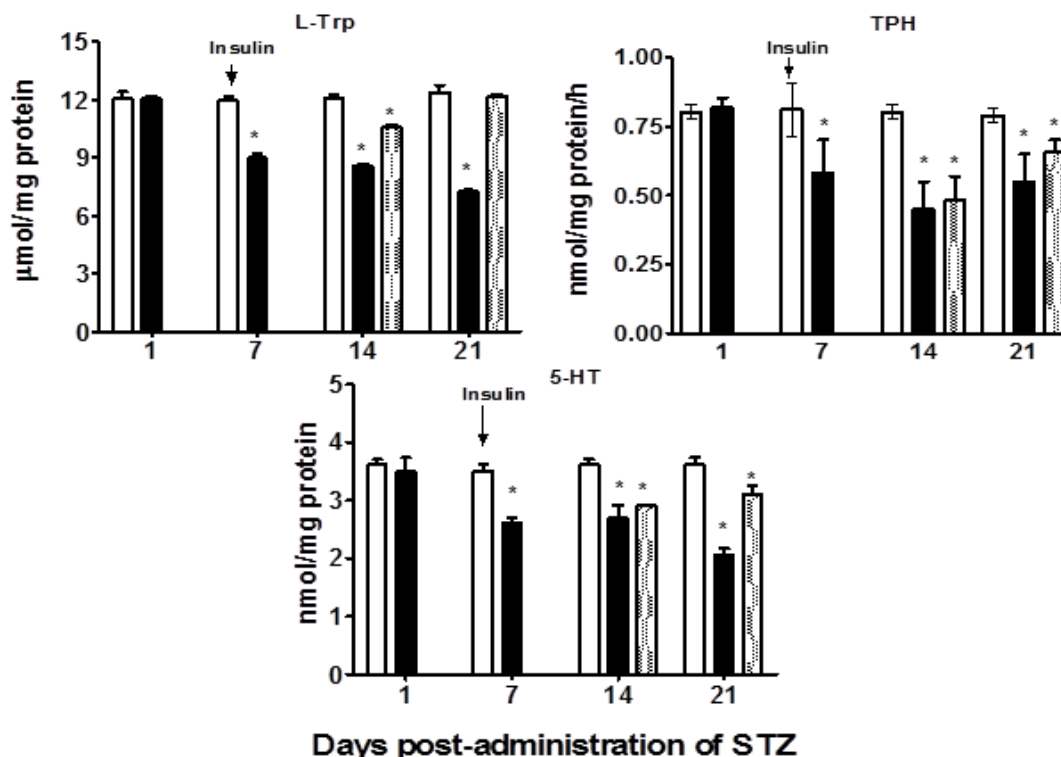


Figure 1. Serotonergic activity in the brainstem of rats. □ Controls; ■ diabetics and ▨ insulin treated diabetics from day 7. Each bar represents the mean value ± SD from twenty rats. All determinations were performed in duplicate samples. The differences between groups was provided by ANOVA (Treatment: Df = 8, SS = 55.46, MS = 10.465, F = 150.6. Time: Df = 7, SS = 0.28, MS = 9.78, F = 0.421) and Student's *t*-test. * *p* < 0.001.

RESULTS

Since the seventh day of the administration of STZ, the blood glucose significantly increased in group D (*p* < 0.001), and as expected the increase was more important on day 21 of the evolution of the diabetic state (*p* < 0.001). Interestingly, the diabetic rats that received insulin achieved normalization of glycaemia after seven days of insulin treatment (Table 1).

It was confirmed that the diabetic rats which were not treated with insulin had a significant decrease in the concentration of L-Trp, TPH activity and 5-HT in the brainstem during the studied period (*p* < 0.001). Another noteworthy point is that in the ITD group, L-Trp levels

increased since day 7 of insulin treatment reaching control values after 14 days. However, in spite of the treatment with insulin, the TPH activity and the 5-HT concentration decreased considerably and did not return to normal values (*p* < 0.001). (See Figure 1)

Figure 2A shows representative kinetic curves of TPH from all groups, at day 14 and 21, after STZ administration, with their respective double reciprocal plots and their corresponding kinetic constants (K_m and V_{max}). According to previous observations (Herrera et al., 2005), the K_m values of TPH in the brainstem of diabetic rats were significantly augmented compared to the C group (*p* < 0.001) (Figure 2B). It is remarkable that the K_m values of the insulin treated diabetic group showed a

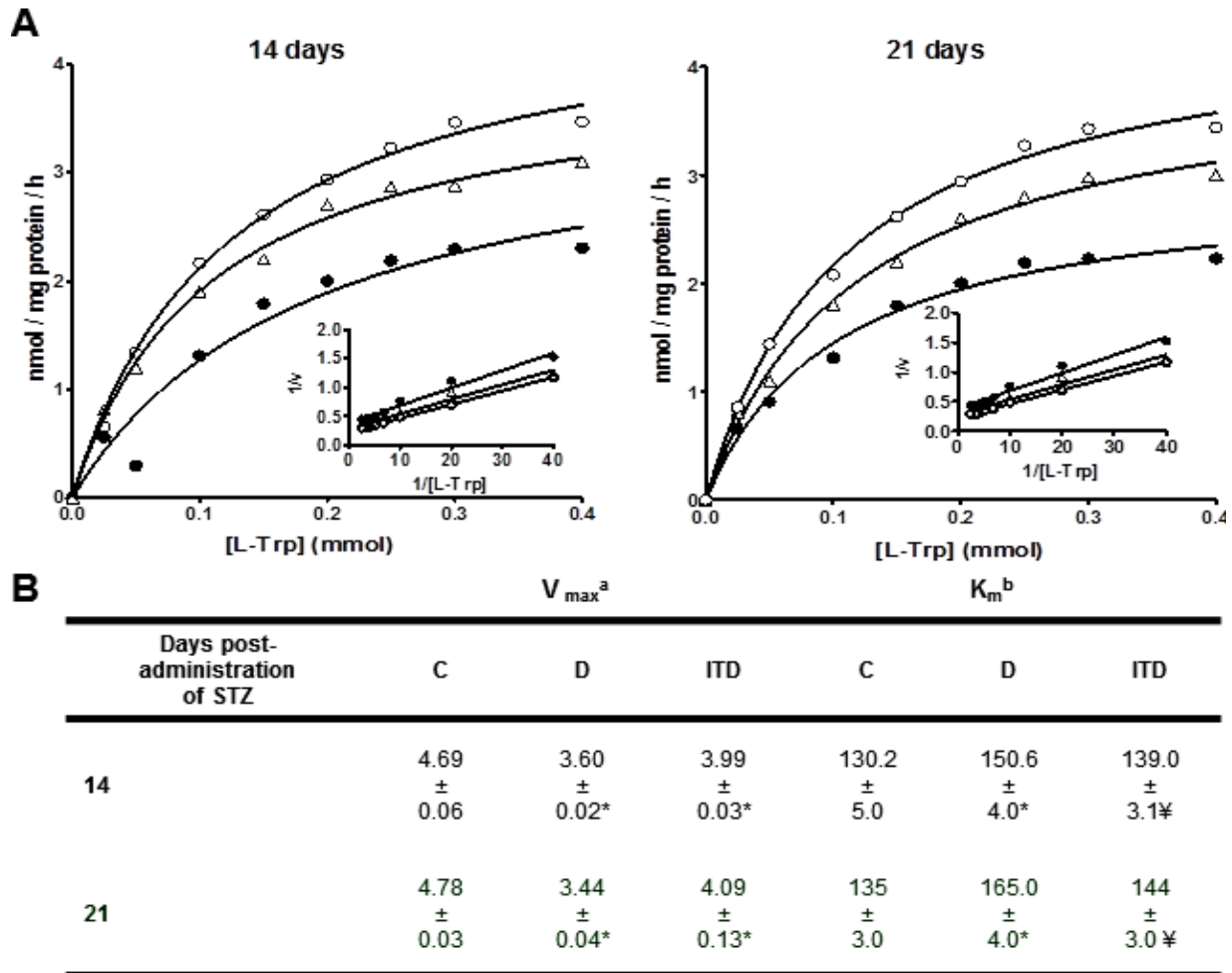


Figure 2. A) Kinetic curves and double reciprocal plots of TPH in the brainstem of rats, days 14 and 21 post-administration of STZ. O, C, controls; ●, D, diabetics; Δ, ITD, insulin treated diabetics. **B)** Kinetic constants of the TPH. Each value represents the mean ± SD (^a nmol of 5-HTP/mg protein/h and ^b μMol) from seven rats. All determinations were performed in duplicate samples. The differences between groups was provided by ANOVA (Df = 7, SS = 62.66, MS = 8.34, F = 150.6. Time: Df = 9, SS = 0.19, MS = 7.78, F = 0.51) and Student's *t*-test. ‡ *p* < 0.05; * *p* < 0.001.

tendency to return to the original state, however they did not reach control values ($p < 0.001$). Likewise, V_{max} values in the brainstems measured in the D group were meaningfully decreased while compared to controls ($p < 0.001$). It is important to mention that the V_{max} in the ITD group showed a tendency to surge, and became significantly higher than the V_{max} values in the non treated animals, but it remained noticeably lower than those found in control groups ($p < 0.001$).

The activity of TPH in phosphorylating conditions (ATP, Mg^{2+} , Ca^{2+} , and d-AMPC) in the brainstem is shown in Figure 3. Both groups, C and D, showed a significant escalation in relation to original values with both activation systems ($p < 0.001$); however, remarkably the phosphorylated enzyme in the diabetic group had a significant less response although it passed basal control values ($p < 0.001$). A similar effect was observed under phosphorylating conditions in the ITD group. The enzyme

activity was also significantly less activated than the C group, and did not reach a comparable response, thus behaving similarly as the non-treated group ($p < 0.001$).

Expressions of TPH1, TPH2 and TPH2-^PS-19 in brainstem by Western blot are shown in Figure 4. There were two bands in all groups (C, D and ITD), on days 14 and 21 after STZ administration, one of 51 kDa for TPH1 and others of 56 kDa which corresponded to the TPH2 and TPH2-^PS-19 (see figure 4A). It was noted that the diabetic group showed a significant decline in the expression of all isoforms of TPH in comparison to the control group ($p < 0.01$). Markedly, the DTI group had a significant increase in the expressions of both isoforms ($p < 0.05$) compared to the D group without insulin treatment. But the expression of all isoforms (TPH1, TPH2 and TPH2-^PS-19) did not reach the values of the C group ($p < 0.001$). (See figure 4B,C and D)

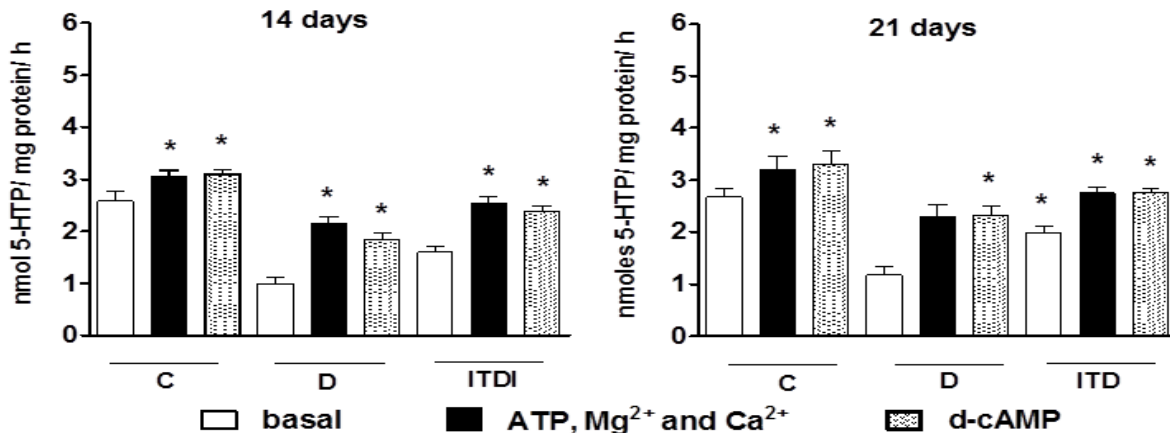


Figure 3. Activation of TPH by ATP, Mg²⁺ and Ca²⁺ and d-AMPC). □ Controls; ■ diabetics and ▨ insulin treated diabetics. Each bar represents the mean ± SD from seven rats, 14 and 21 days post-administration of STZ. All determinations were performed in duplicate samples. The differences between groups was provided by ANOVA (Treatment: Df = 8, SS = 65.56, MS = 9.365, F = 140.6. Time: Df = 9, SS = 0.18, MS = 8.78, F = 0421) and Student's *t*-test. * *p* < 0.001.

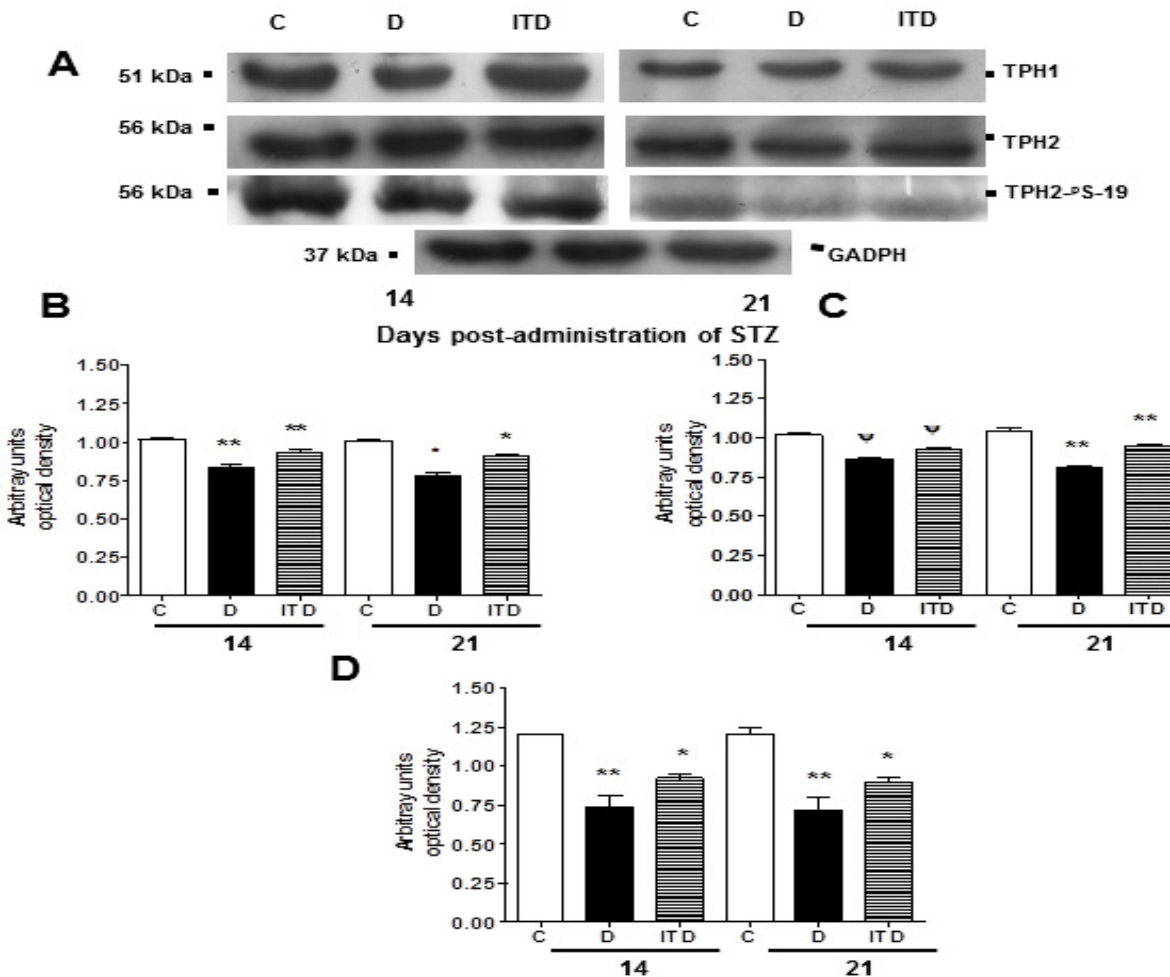


Figure 4. Expression of TPH1, 2 y 2^{-P}S-19 in the brainstem of rats. C, Controls; D, diabetics and ITD, insulin treated diabetics. A) Detected by electrotransference with specific antibodies for each isoform. Three bands were observed, one of 51 kDa (TPH1), other of 56 kDa (TPH2) and 56 kDa (TPH2-^PS-19) and 37 kDa to GADPH as an internal control. B, C and D) Relative optical density of each isoforms. Each bar corresponds to mean values ± SD of six experiments in duplicate samples. The differences between groups was provided by ANOVA (Treatment: Df = 6, SS = 60.10.46, MS = 8.16, F = 120.6. Time: Df = 7, SS = 0.28, MS = 9.78, F = 0.421) and Mann-Whitney U test. * *p* < 0.05, ** *P* < 0.01, ^ψ *P* < 0.001

DISCUSSION

The aim of this study was to determine if the biochemical changes involved in the mechanism of inhibiting the TPH in the diabetic brain, such as: kinetic changes and phosphorylation capacity, remained or were normalized under treatment with insulin; or if there were changes in the expression of TPH1, TPH2 and TPH2^{-PS-19} in serotonergic neurons as possible mechanisms that would explain the chronic inhibition of brain serotonin biosynthesis during the diabetic state. The present results confirm and extend the knowledge about the kinetic behavior of TPH under different conditions of phosphorylation in the brainstem of the diabetic rat without insulin; asserting a decrease in the affinity for its substrate, L-Trp, as well as a decrease in V_{max} . It was also confirmed that under various conditions of phosphorylation, there is a positive response in the control groups, but there is a limitation of its capacity to respond to the phosphorylating stimulus in the diabetic brainstem, despite the insulin treatment (Manjarrez et al., 1999; 2000; Herrera et al., 2005). The present results validate that a second messenger such as cAMP, IP3 and the diacylglycerol are also involved in the activation of the TPH (Johansen et al., 1995; 1996; Kuhn and Arthur, 1997; Boadle-Biber, 1980; Hamon et al., 1977; Ehret et al., 1989). Therefore, all these findings together support the fact that DM produces a major change in the enzymatic behavior due to the modifications in the mechanisms of cellular phosphorylation (Bhardwaj and Kaur 1999; Voitenko et al., 1999; Biessels et al., 2002).

Another point of interest is the biological process that could also explain the inhibition and the TPH kinetic alterations in insulin therapy-resistant diabetic tissues which show different changes in the expression of the isoforms. Both isoforms share homology in sequence and domain structure. Their expression patterns in different tissues show the possibility that their catalytic function are differentially regulated (Walther et al., 2003a; 2003b; McKinney et al., 2005).

So far there are no published records comparing TPH1, TPH2 and TPH2^{-PS-19} expressions in serotonergic neurons in the raphe nuclei during the evolution of the diabetic state. In this study we assessed and compared the expression of both TPH isoforms in the brainstem of untreated diabetic rats and those treated with insulin, comparing to control rats; both isoforms were expressed in all groups. The expression of said isoforms, 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. The present findings, indicate 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 is 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 (Abumaria et al., 2006; 2007; 2008), with a negative impact on the biosynthesis and functionality of this important neurotransmitter and cerebral neuromodulator.

Another mechanism that could also explain the reduced expression of the isoforms, could be explained through their exposure to oxidation conditions (Kuhn and Arthur, 1997a; 1977b; Kuhn and Geddes, 1999) and the increased glucose concentration in the brain (Sima et al., 2004; Brands et al., 2004), causing disorders in the activity of protein kinase A and C (Ehert et al., 1989; Biessels et al., 2002) by decreasing the activity of calcium/calmodulin dependent protein kinase II (Bhardwaj and Kaur 1999) which can rapidly influence the catalytic function of the TPH as it was observed in this work.

Another mechanism that would further explain the reduction in the expression of the isoforms would be a direct damage to the serotonergic neurons of the brainstem by an apoptosis process induced during the diabetic state. These aspects are currently being assessed in our laboratory and require further research.

Therefore, these results represent an example of the induction of an epigenetic modification on a functional protein system: where TPH is modified in an insulin resistant DM. Possibly the changes of enzyme kinetics and its phosphorylating capacity are secondary to those of the TPH molecular complex during the disease, with an impact on the biosynthesis of an important brain neurotransmitter that may be of relevance in the pathophysiology of anxiety and depression in diabetic patients (Hermanns et al., 2005; Manjarrez et al., 2009).

ACKNOWLEDGMENTS

This research was supported by grants from Mexican Institute of Social Security (IMSS). (FIS/IMSS/PROT/G10/882)

REFERENCES

- Abumaria N, Riic A, Anacker Ch, Fuchs E, Flügge G (2008). Stress upregulates TPH1 but not TPH2 mRNA in the rat dorsal raphe nucleus: identification of two TPH2 mRNA splice variants. *Cell. Mol. Neurobiol.* 28:331-342.
- Abumaria N, Rygula R, Havemann-Reinecke U, Rüter E, Bodemer, W, Roos, et al (2006). Identification of genes regulated by chronic social stress in the rat dorsal raphe nucleus. *Cell. Mol. Neurobiol.* 26:145-162.
- Abumaria N, Rygula R, Hiemke C, Fuchs E, Havemann-Reinecke U, Rüter E, et al (2007). Effect of chronic citalopram on serotonin-related and stress-regulated genes in the dorsal raphe nucleus of the rat. *Eur. Neuropsychopharmacol.* 17:417-429.

- Biessels JG, TerLaak PM, Hamers PTF, Gispen HW (2002). Neuronal Ca^{2+} dysregulation in diabetes mellitus. *Eur. J. Pharmacol.* 447:201-209.
- Boadle-Biber MC (1980). Activation of tryptophan hydroxylase from slices of rat brain stem incubated with N^6, O^2 -dibutyryl adenosine-3':5'-cyclic monophosphate. *Biochem. Pharmacol.* 29:669-672.
- Brands MAA, Kessels PCR, de Haan HFE, Kappelle JL, Biessels JG. (2004). Cerebral dysfunction in type 1 diabetes: effects of insulin, vascular risk factors and blood-glucose levels. *Eur. J. Pharmacol.* 490:159-168.
- Ehret M, Cash ChD, Hamon M, Maitre M1(989). Formal demonstration of the phosphorylation of rat brain tryptophan hydroxylase by Ca^{2+} /Calmodulin-dependent protein kinase. *J. Neurochem.* 52:1886-1891.
- Fitzpatrick PF (2000). The aromatic amino acid hydroxylase. *Adv. Enzymol. Relat. Areas Mol. Biol.* 74:235-294.
- Grahame-Smith DG (1964). Tryptophan hydroxylation in brain. *Biochem. Biophys. Res. Commun.* 16:586-592.
- Hamon M, Bourgoin S, Hery F, Simonnet G (1977). Activation of tryptophan hydroxylase by adenosine triphosphate, magnesium and calcium. *Mol. Pharmacol.* 14:99-110.
- Herrera MR, Hernandez-RJ, Medina SJ, Boyzo MOA, Manjarrez GG (2011) Association of metabolic syndrome with reduced central serotonergic activity. *Metab. Brain Dis.* 26:29-35.
- Herrera MR, Manjarrez GG, Hernández RJ (2005). Inhibition and kinetic changes of brain tryptophan-5-hydroxylase during insulin-dependent diabetes mellitus in the rat. *Nutr. Neurosci.* 8:57-62.
- Herrera MR, Manjarrez GG, Nishimura E, Hernandez RJ (2003). Serotonin-Related Tryptophan in Children with Insulin-Dependent Diabetes. *Pediatr. Neurol.* 28:20-23.
- Hufton SE, Jennings IG, Cotton RGH (1995). Structure and function of the aromatic amino acids hydroxylase. *Biochem. J.* 311:353-366.
- Jequier E, Robinson DS, Lovenberg W, Sjoerdsma A (1969). Further studies on tryptophan hydroxylase in rat brainstem and beef pineal. *Biochem. Pharmacol.* 18: 1071-1081.
- Johansen PA, Jennings I, Cotton RGH, Kuhn DM (1995). Tryptophan hydroxylase is phosphorylated by protein kinase A. *J. Neurochem.* 65:882-887.
- Johansen PA, Jennings I, Cotton RGH, Kuhn DM (1996). Phosphorylation and activation of tryptophan hydroxylase by exogenous protein kinase A. *J. Neurochem.* 66:817-823.
- Kaufman S (1985). Aromatic amino acid hydroxylases. *Biochem. Soc. Trans.* 13:433-437.
- Kuhn DM, Arthur Jr RE (1997). Molecular mechanism of the inactivation of tryptophan hydroxylase by nitric oxide: attack on critical sulfhydryls that spare the enzyme iron center. *J. Neurosci.* 17:7245-7251.
- Kuhn DM, Arthur Jr RE (1997). Inactivation of tryptophan hydroxylase by nitric oxide: enhancement by tetrahydrobiopterin. *J. Neurochem.* 68:1495-1502.
- Kuhn DM, Arthur Jr RE, States JC (1997). Phosphorylation and activation of brain tryptophan hydroxylase: identification of serine-58 as a substrate site for protein kinase A. *J. Neurochem.* 68:2220-2223.
- Kuhn DM, Geddes TJ (1999). Peroxynitrite inactivates tryptophan hydroxylase via sulfhydryl oxidation. *J. Biol. Chem.* 274:29726-29732.
- Manjarrez GG, Herrera MR, Leon M, Hernández RJ (2006). A low brain serotonergic neurotransmission in children with type 1 diabetes detected through the intensity dependence of auditory evoked potentials. *Diabetes Care* 29:73-77.
- Manjarrez GG, Vázquez F, Delgado M, Herrera MR, Hernández RJ (2007). A Functional Disturbance in the Auditory Cortex Related to a Low Serotonergic Neurotransmission in Woman with Type 2 Diabetes. *Neuroendocrinol.* 86:289-294.
- Manjarrez GG, Gonzalez RM, Boyzo MOA, Hernandez RJ (2010). Dihydropteridine reductase activity in the brainstem of intrauterine growth-restricted rats. *Int. J. Dev. Neurosci.* 28:621-634.
- Manjarrez GG, Herrera MJR, Bueno SS, González RM, Hernández RJ (2000). Cambios en la biosíntesis de serotonina cerebral en ratas con diabetes mellitus inducidos por estreptozotocina: Efecto del tratamiento con insulina. *Rev. Invest. Clin.* 52:509-516.
- Manjarrez GG, Herrera MJR, Molina HA, Bueno SS, González RM, Hernández RJ (1999). Alteraciones de la neurotransmisión serotoninérgica cerebral inducidas por diabetes mellitus tipo 1. *Rev. Invest. Clin.* 51:293-302.
- Manjarrez GG, Herrera MR, Mejenes SA, Godinez LT, Hernandez-RJ (2009). Functional change of auditory cortex related to brain serotonergic neurotransmission in type 1 diabetes adolescents with and without depression. *World J. Biol. Psychiatry* 10:877-883.
- McKinney J, Knappskog PM, Haavik J (2005). Different properties of the central and peripheral forms of human tryptophan hydroxylase. *J. Neurochem.* 92:311-320.
- Moran GR, Daubner SC, Fitzpatrick PF (1998). Expression and characterization of the catalytic core of tryptophan hydroxylase. *J. Biol. Chem.* 273:12259-12260.
- Naish JS, Boenisch AJ, Farmilio U, Stead RH (1989). *Handbook of Immunochemical Staining Methods.* Dako, Carpinteria, CA.
- Neckers L, Biggio G, Moja E, Meek J (1971). Modulation of brain tryptophan hydroxylase activity by brain tryptophan content. *J. Pharmacol. Exp. Ther.* 177:475-80.
- Peat MA, Gibb JW (1983). High-performance liquid chromatography determination of indoleamines, dopamine, and norepinephrine in rat brain with fluorometric detection. *Anal. Biochem.* 128:275-280.
- Sima AAF, Kamiya H, Li GZ (2004). Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. *Eur. J. Pharmacol.* 490:187-197.
- Veenstra VWJ, Cook EH Jr (2003). Knockout mouse points to second form of tryptophan hydroxylase. *MolInterv.* 3:72-75.
- Walther DJ (2003). Synthesis of serotonin by second tryptophan hydroxylase isoform. *Science.* 299: 76.
- Walther DJ, Bader M (2003). A unique central tryptophan hydroxylase isoform. *Biochem. Pharmacol.* 66:1673-1680.