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Research report

Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes

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Abstract

The influence of diabetes mellitus on brain pathology is increasingly recognized. Previous contributions of our laboratory demonstrated in models of type 1 diabetes (nonobese diabetic and streptozotocin (STZ)-treated mice), a marked astrogliosis and neurogenesis deficit in hippocampus and increased expression of hypothalamic neuropeptides. In the present investigation, we further analyzed alterations of astroglia and neurons in the hippocampus of mice 1 month after STZ-induced diabetes. Results showed that these STZ-diabetic mice presented: (a) increased number of astrocytes positive for apolipoprotein-E (Apo-E), a marker of ongoing neuronal dysfunction; (b) abnormal expression of early gene products associated with neuronal activation, including a high number of Jun + neurons in CA1 and CA3 layers and dentate gyrus, and of Fos-expressing neurons in CA3 layer; (c) augmented activity of NADPH-diaphorase, linked to oxidative stress, in CA3 region. These data support the concept that uncontrolled diabetes leads to hippocampal pathology, which adjoin to changes in other brain structures such as hypothalamus and cerebral cortex.

Theme: Endocrine and autonomic regulation
Topic: Neuroendocrine regulation: other

Keywords: Diabetes mellitus; Hippocampus; Apolipoprotein-E, Fos, Jun, NADPH-diaphorase; Neurodegeneration; Streptozotocin; Astrocytes; Neurons

1. Introduction

Diabetes mellitus, regardless of its type, is associated with cerebral alterations in both human and animal models of the disease [5,23,44]. These alterations include abnormal expression of hypothalamic neuropeptides [11,57], hippocampal astrogliosis [59], decreased hippocampal synaptic plasticity [34,41], neurotoxicity, and changes in glutamate neurotransmission [8,20,67]. Diabetic patients are prone to moderate alterations in memory and learning [18,61] which occasionally may become severe [24] and to enhanced risk of stroke, depression, dementia, and Alzheimer's disease (AD) [3,17,23,28,43,44,48,49,65]. Recently, we have established that neurogenesis, the proliferation of new neurons in the adult brain, is strongly reduced in STZ-treated mice. Furthermore, this deficit has been successfully prevented by administration of estrogens to diabetic mice [60]. Hippocampal neuronal loss by apoptosis is present in animals suffering from long-term spontaneous diabetes (BB/W) in particular if complicated with ischemia [39,53]. However, such damage in the absence of appropriate treatment is not exclusive of hippocampus, since lesions and functional alterations of the

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cerebral cortex and hypothalamus have also been reported [2,11,48,57,62].

We previously found in two animal models of type 1 diabetes (T1D) an increased number of glial fibrillary acidic protein (GFAP)-reactive astrocytes in the hippocampal stratum radiatum [59]. These animal models include firstly the nonobese diabetic (NOD) mouse, which develops spontaneously the disease permitting analysis during the prediabetic and diabetic period. Secondly, the STZ-induced model, where changes are assessed after onset of the diabetes. In the present study, the STZ model is used for further study of markers for changes in astrocytes and neurons.

Astrocytosis is often related to neurodegenerative diseases and aging, in which neuronal dysfunction or damage can also be found [14,25,42,56]. Apo-E, an apolipoprotein primarily localized in astrocytes [19], plays a major role in various CNS disorders, particularly AD. Apo-E, involved in metabolism and lipid transport, is one of the amyloid-related proteins of AD cerebral amyloid plaques [30,54,66]. The Apo-Eε4 genotype is associated with a specific cognitive disadvantage in young women with T1D [16]. Moreover, individuals with both type 2 diabetes and the Apo-Eε4 allele show increased risk for AD [52]. Degenerating neurons can also express Apo-E after kainic acid treatment, while mild neuronal degeneration correlates with increased Apo-E levels in hippocampal astrocytes [26]. It should be mentioned, however, that Apo-E also is implicated in synaptic plasticity and learning [74]. Its accumulation in neurons following injury has been associated with neuronal survival [6].

Early gene products, such as the proteins encoded by proto-oncogenes of the c-fos and c-jun families, are the major components of the transcription factor activator protein 1 (AP-1), where they form different hetero- or homodimers. Suggestions have been presented that, in the CNS, these proteins may be involved in cell proliferation, gene transcription, stress response, regeneration, and cell death [46,55,63,75]. In the hypothalamus of diabetic rats, increased neuronal activity is observed in paraventricular nucleus (PVN), supraoptic nucleus (SON), and median preoptic nucleus (MnPO) measured by Fos specific staining [76]. On the one hand, there are evidences for an association between immediate early gene products and apoptosis in different experimental models, including cerebral hypoxic-ischemic insult or injury [29,73]. On the other hand, c-Jun expression has been associated with neuronal survival [12,38,76]. Finally, some authors proposed c-fos and c-jun products as markers of a hippocampal subpopulation of neurons sensitive to excitotoxicity rather than as predictors of cell survival or death [55].

Another predictor of neuronal function is NO, formed during the conversion of L-arginine in a NADPHd-dependent reaction by NO synthase (NOS) acting as an important intracellular signaling molecule [21]. NADPH-d is recognized as a histochemical marker for NOS [36,58]. Its neuronal isoform, nNOS, is found in neuronal cell bodies, dendrites and axons [7]. In the CNS, NO plays a major role in neuronal development and maturation [50]. Under aging or pathological conditions, such as neurodegenerative diseases, brain injury, or damage, there is increased NO production, with deleterious effects on neuronal function via enhanced oxidative stress.

Accordingly, the present study was designed to further asses the damage inflicted to neurons and astrocytes by experimental diabetes. For this purpose, three representative markers of neurodegeneration and neuronal dysfunction were measured in the hippocampus of mice with STZ-induced diabetes. These markers include the number of Apo-E immunopositive cells as a measure for astrogliosis and neurodegeneration, the level of immunoreactive immediate early gene products for the extent of neuronal activation and the activity of NADPH-diaphorase for the state of oxidative stress.

2. Materials and methods

2.1. Animals and treatment

12-week-old C57BL/6 female mice were housed under conditions of controlled humidity and temperature (22 °C), with lights on from 07:00 to 19:00 h at the facility of the Institute of Biology and Experimental Medicine, Buenos Aires. Experimental procedures followed the NHI Guide for the Care and Use of Laboratory Animals (Assurance Certificate #A5072-01). Mice received a single i.p. dose of 200 mg/kg STZ (Sigma, St. Louis, MO, USA) in 0.5 M sodium citrate buffer or vehicle. Two days after injection, glycosuria was determined using Keto-Diastix (Bayer Diagnostics, Argentina). Following a positive urine test, mice were bled by retro-orbital puncture and blood glucose levels were evaluated using Accutrend (Roche Diagnostics Mannheim, Germany), and quantitatively measured using colorimeter (Accutrend GC, Boehringer Mannheim, Germany). Animals with glycemia higher than 11 mM were classified as overtly diabetic. One month after STZ or vehicle injection, 16-week-old animals were used for immunocytochemistry and NADPH diaphorase (NADPH-d) histochemistry.

Mice were anesthetized with Ketamine (60 mg/kg i.p.) and perfused intracardially with 0.9% NaCl, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB) pH 7.2. Brains were removed, fixed in the same fixative during 4–6 h, incubated overnight in 15% sucrose in PB at 4 °C, frozen on dry ice, and stored at −80 °C until use. Coronal sections (s) (16–30 μm) containing hippocampus [51] were made in a cryostat, mounted on gelatin-coated slides and refixed by immersion in 2% w/v paraformaldehyde in PB during 6 min at 4 °C and washed twice with PB for 10 min.

2.2. Apolipoprotein-E immunocytochemistry

The s (30 μm) were washed in 0.05 M Tris-buffered saline (TBS), preincubated in 10% (v/v) goat serum for 1 h at room

temperature (RT) and incubated overnight at 4 °C with the primary Apo-E polyclonal antibody made in rabbit (kindly provided by Dr. NJ Pearce, SmithKline Beecham, UK, see [26]) in 1/5000 dilution in TBS 0.2%, Triton-X 100, 2% goat serum. After several washes with TBS, the s were incubated in goat anti-rabbit serum (1/200) in TBS 0.2% Triton X-100 for 2 h and processed following the ABC kit (Vector Labs., CA, U.S.A.) instructions. 3,3′-diaminobenzidine (DAB) was used for development at 0.5 mg/ml, 0.05% H_2O_2 at RT. After dehydration with graded ethanols and xylene, the s were mounted with Permount. Nonspecific staining was assessed in the absence of primary antibody; the specific labeling was characterized as mentioned elsewhere [26]. The number of cells expressing Apo-E per area (65×10^3 μm^2) was determined in the stratum radiatum below the CA1 subfield of the dorsal hippocampus using computerized image analysis. Labeled cells were investigated in the mentioned area of both hippocampal sides from five to six s per animal, using five animals per group. Positive cells were identified as reactive astrocytes, based on co-localization between glial fibrillar acid protein (GFAP) and Apo-E by immunocytochemistry and posterior analysis by confocal microscopy (results shown in supplementary data).

2.3. Early genes products (Fos and Jun) immunocytochemistry

Briefly, the protocol used was as follows: brain s were incubated with Fos H-125 polyclonal rabbit antibody (Santa Cruz, Biotechnology, San Diego, CA, USA) 1/3000 in phosphate buffer saline (PBS) 0.15% Triton X-100 overnight at RT or Jun H-79 polyclonal rabbit antibody (Santa Cruz Biotechnology, USA) 1/500 in the same buffer, overnight at 4 °C. After two washes with PBS, s were incubated with goat anti-rabbit serum (1/200) in PBS 0.15% Triton X-100 during 1 h at RT, and processed following the ABC kit instructions (Vector Laboratories, Elite, ABC reagent). Development was carried out using 1 mg/ml DAB 0.01% H_2O_2 during two min at RT. Finally, s were dehydrated in graded ethanols and xylene and mounted with Permount. To assess nonspecific staining due to the immunoprocedure, some s were incubated replacing the primary antibody by no immune rabbit serum. Cells exhibiting Fos and Jun immunolabeling were identified as neurons based on their immunocytochemical reactivity towards the marker somatostatin. Although somatostatin is not an universally accepted marker for neurons, it has been used to stain hippocampal neurons [1]. In this case, somatostatin-immunoreactive cell area (65.9 ± 4.1 μm^2, $n = 21$), was identical to cell area of Fos/Jun-positive cells counterstained with cresyl violet (63.3 ± 2 μm^2, $n = 19$).

2.4. NADPH-diaphorase histochemistry

A slight modification of the method of Vincent and Kimura [71] was employed to determine NADPH-d activity as previously described [36,58]. The latter was used as a marker for NOS, because while neurons can express several NOS isoforms during pathological conditions, all isoforms share NADPH diaphorase activity [69,70]. Cryostat s (16 μm) were incubated in a solution of 0.1M Tris HCl buffer pH 7.4 containing 0.3% (v/v) Triton X-100, 0.2 mg/ml of nitroblue tetrazolium, 2.7 mg/ml l-malic acid and 1 mg/ml ß-NADPH. After 60–90 min at 37 °C in the darkness, the reaction was stopped by two washes in PBS at RT. s were then dehydrated briefly in ethanol, dried and coverslipped with Permount. To assess non-specific staining due to the procedure, some s were incubated in buffer without ß-NADPH. NADPH-diaphorase active cells were identified as neurons based on immunoreaction with an anti-somatostatin antibody, as shown above for Fos and Jun-expressing cells.

2.5. Measurement of hippocampal volume

Cresyl-violet stained serial s (50 μm, every 8 s), obtained with a vibratome, were used to estimate the volume using a computer-assisted image analysis (Optimas Bioscan 4.2) on the basis of the Cavalieri principle. Structures were outlined, and the computed areas were summed and multiplied with the inter distance and with the thickness of the s. Volumes are expressed as mm^3 per 750 μm.

2.6. Computerized image analysis

A computerized image analysis (program Bioscan Optimas II; Edmonton, WA, USA) equipped with a VT-C330N video camera was used for quantitative analysis [59]. Digitized images of tissue s containing hippocampus were displayed on the video screen under identical lighting conditions. Using this program, we set up a threshold for positive cell area, and within these area limits, nucleated cells exhibiting Apo-E labeling were selected for our study. For early genes products, we quantified the number of immunoreactive nuclei in CA1, CA3 and dentate gyrus (DG) areas. For NADPH-d labeling, we quantified the intensity (as inverse logarithm of gray intensity per area, ILIGV/area: LIGV) of the reaction in CA1 and CA3 subfields [36].

2.7. Statistics

Statistical analysis was performed using Student's t test with two-tailed P value. Differences are considered significant when the two-tailed P value is <0.05.

3. Results

3.1. Pathophysiology

The animals were used 4 weeks after STZ injection. At this time period, blood glucose was high and comparable

to animals bled 48 h after diabetes induction. Diabetic mice showed marked hyperglycemia (15.8 ± 2.0 mM), polydipsia (control water intake: 3 ± 0.5 ml/day; diabetics 5.2 ± ml/day, $P < 0.05$), and increased food intake (controls: 0.6 ± 0.02 g/day, diabetics: 0.72 ± 0.03 g/day, $P < 0.05$). Initial body weight (experimental day 1) was similar in control (26.5 ± 1.1 g) and diabetic mice (26.1 ± 0.5 g) but controls were significantly heavier than diabetic animals at the conclusion of the experiment (30.0 ± 1g vs. 20.3 ± 1.4 g, $P < 0.01$). Therefore, at the time of killing, STZ-treated mice showed characteristic signs of overt diabetes.

3.2. Hippocampal volume was not altered in diabetic mice

Measurement of hippocampal volume showed no significant difference between control and STZ-induced diabetic mice: controls: 2.203 ± 0.076, diabetic: 2.317 ± 0.051 mm^3 ($n = 5$ animals in each group).

3.3. More Apo-E^+ cells in the hippocampus of STZ-treated diabetic mice

The immunocytochemical features of immunoreactive Apo-E cells present in the stratum radiatum of 16-week-old vehicle- or STZ-treated mice, under the CA1 region, are given in Figs. 1A and B, respectively. In both groups of mice, Apo-E immunostaining was localized specifically in cells with a spider-like shape typical of astrocytes, exhibiting a GFAP-positive phenotype (please see Appendix). Moreover, Apo-E^+ cells appear more numerous in the STZ-than in the vehicle-treated group. We therefore quantified the number of Apo-E^+ cells in both groups of mice by computerized image analysis (Fig. 2). One month after STZ treatment, the number of Apo-E^+ astrocytes was significantly increased in the STZ-treated group as compared to the vehicle-treated group ($P < 0.001$, $n = 5$).

The number of astrocytes immunoreactive for Apo-E was in accordance with data obtained using a GFAP antibody. In this case, a 1.4-fold increase in cell number was obtained for both APO-E positive and GFAP-positive astrocytes in diabetic compared to control mice.

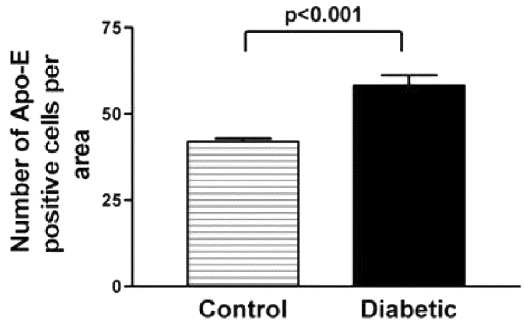

Fig. 2. Quantitation by computerized image analysis using Optimas Bioscan Software of the number of Apo-E positive cells per area (65×10^3 μm^2) in hippocampal stratum radiatum region from control C57BL/6 and STZ-diabetic mice.

3.4. Enhanced expression of early gene products (Jun and Fos) in the hippocampus of STZ-treated diabetic mice

Cellular activation was studied by immunocytochemistry using specific antibodies against Jun and Fos. Immunoreactivity for early gene products was localized in neurons but absent from astroglial cells in white matter regions of hippocampus. Fig. 3 shows the photomicrographs corresponding to Jun in the hippocampal CA1, CA3, and DG neurons and to Fos in CA3, respectively. Numerous positive nuclei were observed in each case in the STZ-treated group compared to the vehicle-treated group. Quantitative assessment of these data by computerized image analysis is shown in Fig. 4. When STZ-treated mice were compared to vehicle-treated mice, the numbers of positive nuclei were significantly higher in diabetic mice, as follows: for Jun (Fig. 4, top), in neurons corresponding to the different hippocampal regions studied (CA1, CA3, and DG) ($P < 0.01$ in each case, $n = 6$) and for Fos (Fig. 4 bottom), in CA3 neurons only ($P < 0.05$, $n = 4$) but a tendency toward increased number of Fos$^+$ nuclei was also observed in CA1 and DG subfields in the diabetic

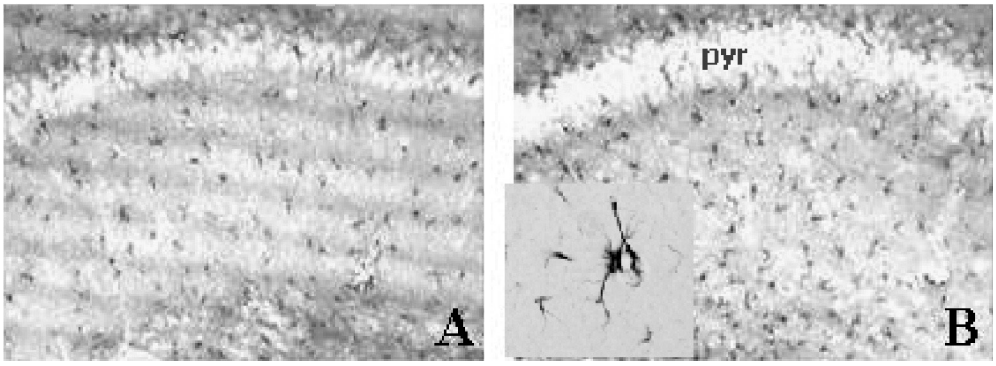

Fig. 1. Photomicrographs showing Apolipoprotein E (Apo-E)-positive cells in hippocampal stratum radiatum from 16-week-old female control C57BL/6 (A), STZ-diabetic mice (B). Note the numerous positive cells in B, pyr: pyramidal cell layer, magnification: 400×. In B, inset showing Apo-E positive cell at higher magnification.

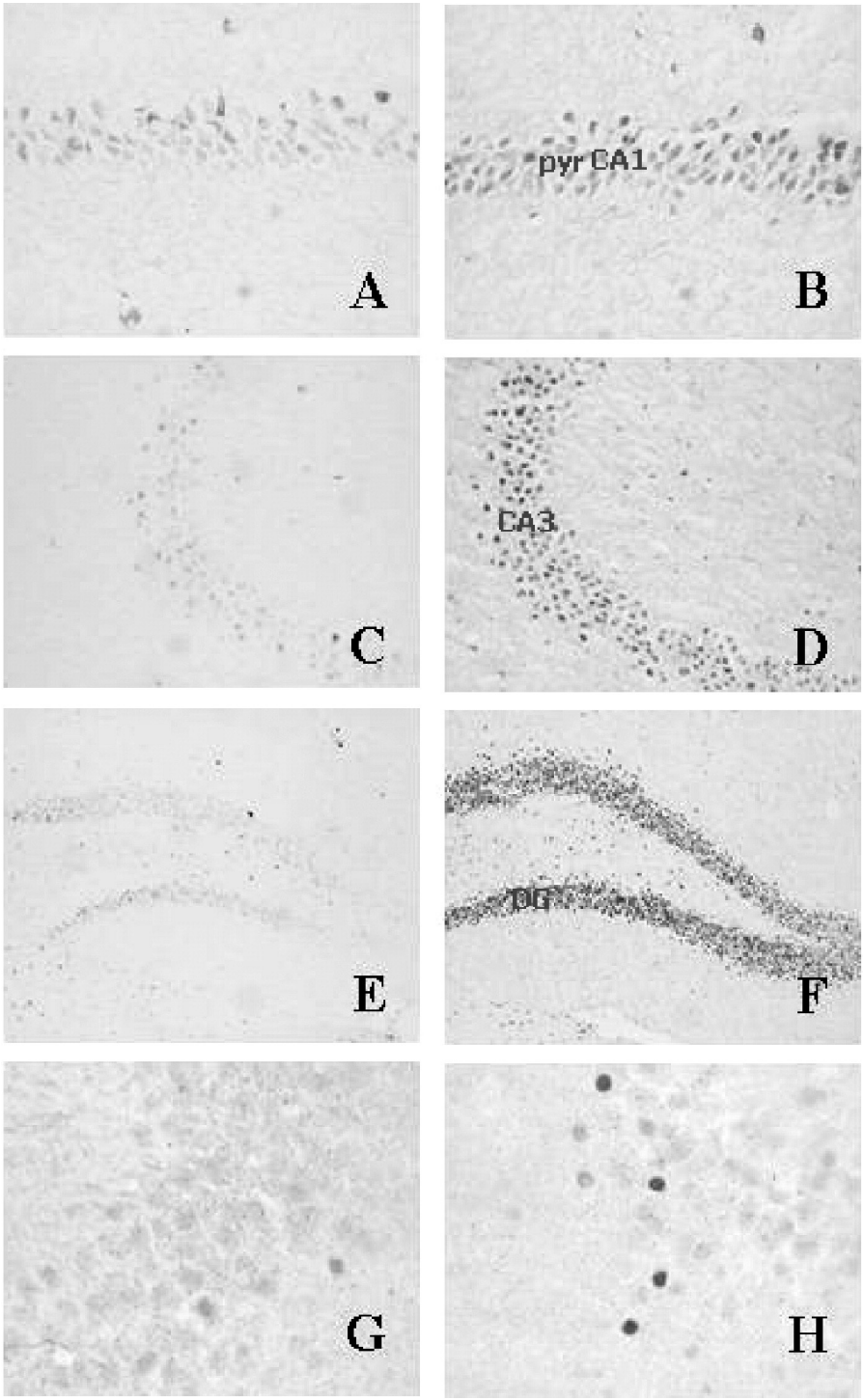

Fig. 3. Photomicrographs showing Jun (A–F) and Fos (G–H) immunoreactive cell nuclei of hippocampal neurons from control (A, C, E, G) and STZ-diabetic mice (B, D, F, H). CA1 area is shown in A, B, CA3 in C, D and G, H, Dentate Gyrus (DG) in E, F photomicrographs. Magnification: A–B: 200×; C–F: 100× and G–H: 400×; pyr CA1: pyramidal cell layer corresponding to CA1 hippocampal region, DG: dentate gyrus.

group. Preliminary data in our short-term model of diabetes showed that the total number of hippocampal neurons in CA1 and CA3 regions presenting somatostatin immunoreaction was similar in control and diabetic mice. Since the presence of both Jun and Fos were linked to development of neurodegeneration [73], we then studied NAPDH-d as a marker of oxidative stress.

3.5. Increased NADPH-diaphorase activity in the hippocampus of STZ-treated diabetic mice

Figs. 5A and B shows an example of NADPH-d histochemistry in the hippocampal CA3 region of both groups of mice. Once again, a marked difference could be observed between both groups, as shown by computerized

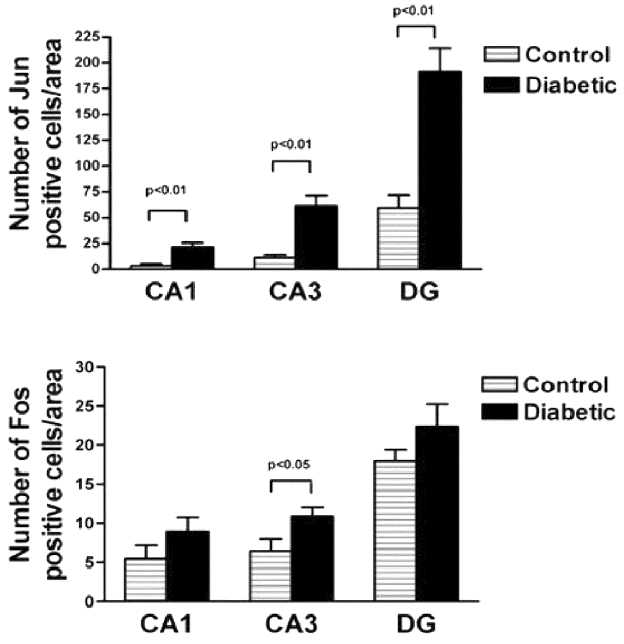

Fig. 4. Quantitation using computerized image analysis of early genes products in hippocampus of control and STZ-diabetic mice. Number of Jun immunoreactive cell nuclei in CA1, CA3 and DG hippocampal regions from vehicle-treated and diabetic STZ-treated mice (top). Number of Fos-immunoreactive cell nuclei in CA1, CA3, and DG hippocampal regions from vehicle-treated and diabetic STZ-treated mice (bottom).

image analysis of staining intensity (Fig. 6). There was a marked increase of enzyme activity in neurons of both the CA1 and CA3 regions, but this difference reached significance only in CA3 ($P < 0.005$, $n = 6$).

4. Discussion

In the present study, some molecular parameters were investigated in the hippocampus of STZ-treated diabetic mice that could reflect functional abnormalities of astrocytes and neurons. Previously, we have reported a substantial reduction of hippocampal neurogenesis in STZ-diabetic mice [60] and increased number of GFAP-immunoreactive astrocytes in the spontaneous model of T1D, the NOD

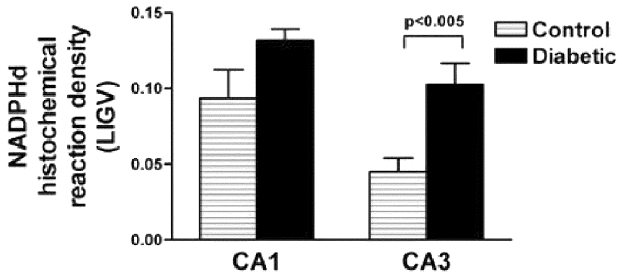

Fig. 6. NADPH diaphorase activity in CA1 and CA3 regions from control and diabetic STZ-treated mice. The histochemical reaction is expressed as relative optical intensity per area (LIGV).

mouse, and the pharmacologically-induced STZ mouse model [59]. Here, we showed, at the astrocyte level, increased Apo-E immunoreactivity in the CA1 region. At the neuronal level, we observed an enhanced expression of the immediate-early gene product Jun in the CA1, CA3, and DG regions of the hippocampus. In addition, a high amount of Fos-positive nuclei and high NADPH-d activity was found in the CA3 region. These findings, as well as previous, indicate that 1-month exposure to uncontrolled diabetes can alter the hippocampus. On the one hand, the alteration might reflect the regenerative capacity of the brain: ApoE, Fos and Jun, and the NO may contribute to the recovery of neuronal processes. On the other hand, the ApoE, Fos, Jun and NO increases may represent responses to a mild neurodegeneration. Below we will discuss the arguments involving these possibilities.

Apo-E is synthesized by astrocytes, oligodendrocytes and ependymal layer cells [6]. It has been suggested that Apo-E has a neurotrophic role that may be needed for synaptic plasticity and learning [74], ApoE also seems involved in the removal of cell debris, because degeneration products were shown to remain in the hippocampus of Apo E deficient-mice [15,68]. Under normal conditions, Apo-E is thought to enter neurons (rather than to be synthesized by them) and this uptake in neurons is enhanced during repair after injury. Peripheral and CNS injury is associated with a strong increase in Apo-E expression in non-neuronal cells [6]. However, after kainic acid-induced hippocampal neurodegeneration, the intensity

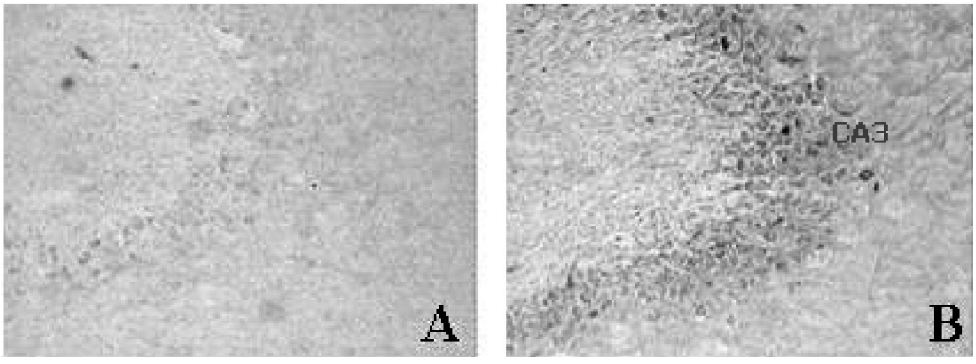

Fig. 5. NADPH diaphorase histochemical staining in CA3 hippocampal region from control (A) and diabetic-STZ treated mice (B). Magnification: 200×.

and cellular distribution of Apo-E is dependent on the severity of neuronal injury, which correlate with the dose of toxin administered. Indeed, mice that developed mild neuronal degeneration restricted to a subset of hippocampal neurons show increased Apo-E expression in the hippocampus concomitant with GFAP immunoreactivity and mild microgliosis. In contrast, mice with severe hippocampal neuronal injury show intense Apo-E expression in degenerating neurons and increased Apo-E mRNA levels in clusters of CA1 and CA3 pyramidal neurons [6,26].

Since no signs of pyramidal cell death are present in Apo-E^{+} neurons of kainic acid-treated mice, it has been suggested that neuronal Apo-E expression may be part of a rescue program to counteract neurodegeneration [6,32]. In humans, Apo-E immunoreactivity has been described in cortical and hippocampal neurons of AD and aged control subjects [27,45]. In the context of this work, it is worth noting the strong association between type 2 diabetes and AD, as well as between type 1 diabetes and changes in cognition among carriers of the Apo-Eε4 allele [16,37,52]. The joint effect of diabetes and Apo-Eε4 is synergistic, causing more than a 5-fold increase in the risk for AD. Therefore, taking into account all these data, it seems likely that an exposure to T1D for 1 month in the STZ-mouse model can induce moderate hippocampal disturbances, since Apo-E expression is increased only at the astrocyte level. The effects on Apo-E expression might reflect a more general effect upon astrocytes, since the total number of these cells, according to GFAP immunocytochemistry, was increased in the stratum radiatum of STZ-diabetic mice [59]. These alterations seem to be early events following diabetes induction, considering that a 30% increase in astrocyte number in the stratum radiatum is measured 48 h after STZ-treatment (unpublished data), which increased to 50% of control levels after 4 weeks of uncontrolled diabetes This is also the case in NOD mice with a genetic type of TD1 [59]. Regarding the significance of increased Apo-E expression, further studies are needed to elucidate if this change reflects an incipient neurodegeneration or represent a response underlying recovery.

In addition to increased astrocyte reactivity [59] and increased Apo-E immunoreactivity in astrocytes (this paper), the induction of T1D also resulted in changes in Jun- and Fos-positive nuclei in neuronal populations of the CA1, CA3 and DG hippocampal areas. Immediate early genes (IEG) products have been extensively investigated in relation to neuronal cell death. In in vitro systems, using NGF-deprived sympathetic neurons IEGs are indicative of neuronal cell death taking place during postnatal development. Cell death linked to IEG production also occurs under in vivo situations after brain injuries, such as hypoxic-ischemia and kainic acid administration [29,31,55,73]. Accordingly, several of these studies show increased expression of Fos and Jun in situations associated with neuronal cell death, aging and impaired cognition [64]. Moreover, the presence of Fos and Jun-like immunoreactivity has been reported in neurons of the AD brain [47]. While these studies imply a functional role of early gene products in neuronal cell death, other studies questioned this interpretation, since many cells that expressed Fos and Jun during progression of excitotoxic lesions due to hypoxic-ischemia or kainic acid administration survive the injury, particularly at the hippocampal level [29,55,73]. It has therefore been suggested that: (1) the biological effect of the Jun N-terminal kinase (JNK)/c-Jun signalling pathway depend on the neuronal type and stage of maturation [13]; (2) Fos and Jun neuronal response might not contribute to cell death but rather to cell repair and/or regenerating processes [29]. Taken together, increased Fos- and Jun-positive cells, particularly in the absence of neuronal Apo-E expression, suggested that neuronal derangement was emerging in the hippocampus of 1-month diabetic animals.

A second neuronal abnormality found in T1D was NADPH-d (or nNOS) which showed a significant increase in the CA3 hippocampal field of STZ-treated diabetic mice. Normally, NADPH-d is present in the intact brain only in selected populations of neurons but absent from glial cells. It is only after a severe brain lesion that astrocytes display intense NADPH-d activity [72]. With regard to diabetes, 4 weeks after STZ injection into rats, increased expression of nNOS mRNAs and NADPH-d staining are found in the hypothalamus, which are partly suppressed by insulin treatment [62]. As discussed by others, the significance of the up regulation of NO during brain lesions is ambiguous. NO could have a protective effect on damaged neurons, being a major factor of synaptogenesis and axonal sprouting and might be associated with neuronal regeneration. But it may also be involved in neuronal cell death [35]. These authors recently showed using nNOS-KO mice that local release of NO following peripheral nerve injury is a crucial factor in degeneration and/or regeneration.

With regard to the STZ effects on the brain, and particularly, on the hippocampus, it has already been stressed that the type and the extent of the alterations are a function of both the severity of hyperglycemia as well as its duration [5]. First, alterations of Apo-E-positive astrocytes, as those described here, may accompany a mild neuronal activation that could reflect a stage of reactivity or adaptation to metabolic disturbances. Concomitantly, the diabetic process can affect neuronal renewal [33]. If the deleterious stimulus continues, then signs of neurodegeneration, alterations of neuronal morphological parameters, and neuronal cell death would occur. In this sense, a time-related apoptosis was observed in a genetic model of T1D, the BB/W rat [39]. These authors showed beside an increased number of TUNEL-positive cells, stimulated levels of caspase-3 activity, internucleosomal DNA cleavage and induction of pro-apoptotic genes in hippocampus of 8-month but not in 2-month diabetic rats.

In addition to hyperglycemia, other disturbances may contribute to the changes observed in the hippocampus. In this respect, it is known that diabetic animals show aberrant activity of the glucocorticoid system, including increased circulating corticosterone levels, lack of periodicity, hypersensitivity to stress, and down-regulation of hippocampal glucocorticoid receptors [10,22,41]. In STZ-diabetic mice and in NOD mice, we have shown increased levels of vasopressin mRNA in the hypothalamic paraventricular nucleus, indicative of persistent stress [57]. Thus, the highly stressful condition of diabetic animals might enhance the vulnerability of brain areas with a high degree of plasticity such as the hippocampus [9]. Therefore, it is likely that anatomical and phenotypical features observed in the hippocampus underlie the impairment of cognitive performance of diabetic animals [3,17,23]. Along this line, preliminary studies demonstrated that the exploratory activity in the closed arm (non-conflicting environment) of the elevated asymmetric plus-maze set up is decreased in STZ-diabetic mice (Saravia et al., unpublished).

The present study completes the view of the hippocampal disturbances that we previously described in T1D mouse model [59,60]. The observed changes are not fully conclusive of hippocampal neurodegeneration. Only after prolonged untreated diabetes severe hippocampal neurodegeneration with signs of apoptosis may develop [39] or appear as secondary complications of the disease [53]. Moreover, the hippocampal disturbances described here and elsewhere after 1 month of diabetes could not a priori be attributed to hippocampal volume changes because no significant differences on volume measurement were found between control and diabetic mice. The fine hippocampal disturbances showed in this model of T1D could be among the primary basic mechanisms underlying the well-known brain alterations associated with diabetes. Such alterations, like the dysregulation of the hypothalamo–pituitary–adrenal axis [10,40,41], increased risk of stroke and dementia [5] and cognitive deficits are also found during ageing. Learning and memory, for example, two processes in which hippocampal synaptic plasticity is involved, can be disturbed in both diabetic humans and animals [4,8,20,34,67]. Moreover, in humans with long-standing diabetes, marked deficits in cognition were also reported [24]. Therefore, data obtained in the mouse hippocampus may be useful to interpret the functional deficits of diabetic patients.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainres.2004.12.032.

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