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Research report

Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice

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Abstract

Diabetes can be associated with cerebral dysfunction in humans and animal models of the disease. Moreover, brain anomalies and alterations of the neuroendocrine system are present in type 1 diabetes (T1D) animals, such as the spontaneous nonobese diabetic (NOD) mouse model and/or the pharmacological streptozotocin (STZ)-induced model. Because of the prevalent role of astrocytes in cerebral glucose metabolism and their intimate connection with neurones, we investigated hippocampal astrocyte alterations in prediabetic and diabetic NOD mice and STZ-treated diabetic mice. The number and cell area related to the glial fibrillary acidic protein (GFAP)-immunoreactive astrocytes were quantified in the stratum radiatum region of the hippocampus by computerized image analysis in prediabetic (2, 4 and 8 weeks of age) and diabetic (16-week-old) NOD female mice, age and sex-matched lymphocyte-deficient NOD*scid* and C57BL/6 control mice and, finally, STZ-induced diabetic and vehicle-treated nondiabetic 16-week-old C57BL/6 female mice. Astrocyte number was higher early in life in prediabetic NOD and NOD*scid* mice than in controls, when transient hyperinsulinemia and low glycemia were found in these strains. The number and cell area of $GFAP^+$ cells further increased after the onset of diabetes in NOD mice. Similarly, in STZ-treated diabetic mice, the number of $GFAP^+$ cells and cell area were higher than in vehicle-treated mice. In conclusion, astrocyte changes present in genetic and pharmacological models of T1D appear to reflect an adaptive process to alterations of glucose homeostasis.

Theme: Other systems of the CNS

Topic: Limbic system

Keywords: Hippocampus; Diabetes mellitus; Astrocyte

1. Introduction

The impact of diabetes mellitus on the central nervous system (CNS) is well recognized. In humans, diabetes is associated with impairment of cognitive performance, risk of stroke, cerebrovascular and Alzheimer disease while morphologically, evidences of modest cortical and subcortical atrophy were found [8,20,27,39,40,42]. In diabetic animals, deficits in cognitive performance may be explained in part by the neurotoxic effects of hyperglycemia [12,19,24,40] and changes in glutamate neurotransmission [13,67]. Evidence for brain disturbances were reported in hypothalamus, cerebral cortex and hippocampus of streptozotocin (STZ)-induced diabetic rats [7,28,36,53]. Diabetic animals also show increased sensitivity to stress, high circulating glucocorticoid levels and down-regulation of

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hippocampal glucocorticoid receptors [8,14,37,49,57,62]. These conditions enhanced the vulnerability to metabolic insults of brain areas showing a high degree of plasticity, such as the hippocampus [14,20]. By contrast, hypoglycemia, can take place during insulin treatment and lead to brain disturbances as well [1,48,70].

Glucose metabolism and its possible disturbances in the CNS are not only important for neurons but also for astrocytes, the most numerous cells in the central nervous system. A role for astrocytes may be envisaged considering that they are a prevalent site of glucose uptake, metabolism and coupling to synaptic activity, due to their intimate connection to intraparenchymal capillaries and neurons [6,38,43,46,68,69]. Astrocyte abnormalities in the hippocampus have been observed during stress, aging, autoimmune and neurodegenerative diseases and in STZ-diabetic rats [31,32,65,66]. In these rats, hippocampal damage, causing hyperreactivity to stress and involving both neurons and astrocytes, has been observed [7,37]. Therefore, astrocytes appear to be a major potential target during abnormal glucose homeostasis including hyper and hypoglycemia.

At variance with pharmacological induced models of T1D, the nonobese diabetic (NOD) mouse, a spontaneous model of the disease [5,56], allows to study changes occurring during both prediabetic and diabetic periods. The prediabetic NOD mouse presents various metabolic, neuroendocrine and behavioral disturbances, including hyperactivity of the hypothalamo–pituitary–adrenal (HPA) axis to IL-1, and increased sensitivity to the behavioral effects of this cytokine [2–4,9,26]. From 12 weeks of age onwards, clinical onset of diabetes may occur due to the autoimmune destruction of pancreatic insulin-producing β cells and consequently the lack of insulin [5,26]. In recent-onset diabetic NOD mice, we reported an increased expression of hypothalamic arginin-vasopressin and oxytocin mRNA [60], a finding in common with STZ diabetic rats and obese db/db mice [15,41]. These neuropeptide changes may reflect a potentially high sensitivity to stress due to diabetes acting as an endogenous metabolic stressor [60]. In this regard, diabetic humans and both NOD mice and STZ-diabetic animals appear to be hypersensitive to stress [2–4,10,14,62].

Our objective was to study astrocyte reactivity in the NOD hippocampus during the prediabetic and diabetic stage and in mice rendered diabetic by STZ treatment. Induction of glial fibrillary acidic protein (GFAP), an astrocyte intermediate filament cytoskeletal protein, is considered the main indicator of astroglial activation caused by CNS injury, aging and neurodegeneration [21,35,44]. Therefore, the number and area of GFAP immunoreactive astrocytes was quantified in hippocampal stratum radiatum at various ages during prediabetes in NOD mice (2-, 4- and 8-week-old), times during which hypoglycemia and hyperinsulinism developed in this strain [3,55]. The same parameters were then assessed after diabetes onset in 16-week-old NOD and in STZ-treated C57BL/6 mice, which were severely hyperglycemic. Thus, we hypothesized that adaptive responses of astrocytes would follow the cycling between hypo and hyperglycemia.

Here we show that hippocampal astrocytosis characterized the hippocampus of the genetic and pharmacological induced models of diabetes.

2. Materials and methods

2.1. NOD mice

NOD originally provided by Clea Japan Inc. (Tokyo, Japan), NOD*scid* (lymphocyte-deficient strain, not developping diabetes) [52] and C57BL/6 mice were bred and kept at the facilities of Hôpital Necker, Paris, France. Female mice were fed standard pellets and water ad libitum and maintained at 22±1 °C on a 12/12 h light–dark cycle, under specific pathogen-free conditions. Animals were handled according to the European Community guideliness [3]. From 10 weeks onwards, NOD females were tested weekly for glycosuria. After a positive test was obtained, animals were bled by retroorbital puncture and blood glucose levels were assessed by Glukotest (Boehringer-Mannheim, Mannheim, Germany). Animals showing blood glucose >11 mM were considered diabetic. In our colony, hyperglycemia routinely appears in some of the NOD females from 12 weeks of age onwards (earlier than in males) and 80% of the females become diabetic at 24 weeks of age (but only 40% of males) [3,26]. In this study, females of the C57BL/6 control strain, NOD and NOD*scid* were used at 2, 4, 8 and 16 weeks of age for GFAP immunohistochemistry. When the experiment was performed in 16-week-old NOD mice, animals were hyperglycemic for 3 weeks. We did not wait for a longer diabetes duration in order to avoid animal death in the absence of exogenous insulin.

2.2. Streptozotocin treatment

Eleven-week-old female C57BL/6 mice bred in La Plata University, Argentina, were housed at the facility of the Institute of Biology and Experimental Medicine under similar conditions shown above for NOD mice. Experimental procedures followed the NIH Guide for the Care and Use of Laboratory Animals. One week after arrival, 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 the injection mice glycosuria was determined using Keto-Diastix (Bayer Diagnostics, Argentina). Following a positive urine test, mice were bled by retroorbital puncture and blood glucose levels were evaluated using Accutrend (Roche Diagnostics, Mannheim, Germany) and quantitatively measured using

colorimetry by 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 GFAP immunohistochemistry. We chose to study diabetic NOD and C57BL/6 mice of the same age, in order to avoid a possible effect of aging on astrogliosis [32], and under almost similar diabetes duration (3–4 weeks, respectively).

2.3. Blood glucose and insulin determinations in NOD strain

Unanesthetized animals were bled in less than 2 min by retro-orbital puncture. As previously shown, this technique avoids stress-induced metabolic changes [16]. Glucose concentrations were measured using the glucose-oxidase method (Biotrol glucose enzymatic color, Biotrol, Paris, France). Plasma insulin concentrations were determined using a standard RIA (SB-insulin-CT, CIS Biointernational, Gif-sur-Yvette, France), as previously described [2–4]

2.4. GFAP immunohistochemistry and morphometric analyses

For immunohistochemical studies, mice were anesthetized with tribromoethanol or 'Avertin' (Aldrich–Chimie, Steiheim, Germany) and perfused intracardially with 0.9% NaCl [60]. Brains were removed and fixed in 10% (v/v) formalin for 48 h and embedded in paraffin. For immunocytochemistry, 5 μm brain sections were washed in phosphate-buffered 0.9% NaCl (PBS), preincubated in 10% (v/v) goat serum, and incubated overnight at 4 °C with a 1/250 dilution of rabbit anti-GFAP polyclonal antibody (G-9269, Sigma) in a moist atmosphere. Following a wash with PBS, the primary antibody bound to the sections was revealed using a biotinylated goat anti-rabbit complex (Vectastain ABC Elite Kit, Vector Labs, Burlingame, CA, USA). For the final reaction products, sections were exposed to 0.25 mg/ml diaminobenzidine tetrachloride and 0.01% (v/v) H_2O_2 for 6–8 min in the dark. The sections were then given a rinse in PBS, dehydrated in graded ethanols and xylene, and mounted with Permount [22].

The number of cells expressing GFAP per 65×10^3 μm^2 and the area (in μm^2) of each positive nucleated cell were assessed in the stratum radiatum below the CA1 subfield of the dorsal hippocampus. Examination was centralized in this area considering its enrichment in GFAP-immunoreactive astrocytes according to Catalani et al. [11]. A computerized image analysis (Bioscan Optimas II, Edmonton, WA, USA) equipped with a VT-C33ON videocamera was used for quantitative analysis [18,60]. Digited images of tissue sections containing hippocampus [47] 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, a nucleated cell exhibiting GFAP labeling was considered for our study. Labeled astrocytes were investigated in the right and left hippocampal sides from five to six sections per animal, using four to six animals per strain/age group.

2.5. Statistical analyses

Data are presented as mean±S.E. Differences in astrocyte number and cell area between control mice, NOD*scid* and NOD nondiabetic or NOD diabetic in each age group and control and STZ-treated mice were analyzed by one-way ANOVA. Where the variance (F) ratio indicated a significant difference ($P<0.05$ or less), group comparisons were analysed by the Bonferroni or Newman-Keuls post-hoc test.

3. Results

3.1. Blood glucose and insulin levels

Data in Table 1 show that basal nonfasting blood glucose levels in NOD mice were lower than in C57BL/6,

Table 1
Basal blood glucose and insulin levels in nonfasting female C57BL/6, lymphocyte-deficient NOD*scid* and prediabetic NOD mice as a function of age

	Age (weeks)			
	2	4	8	16
Glucose (mmol/l)				
C57BL/6	4.7±0.1	7.4±0.25	8.1±0.2	6.4±0.3
NOD*scid*	5.0±0.5	6.1±0.1*	6.4±0.2*	5.9±0.7
NOD	3.8±0.1*	6.9±0.1	6.2±0.3**	6.8±0.5
Insulin (pmol/l)				
C57BL/6	72.4±5.0	118.0±10.0	106.0±7.0	164.8±31.4
NOD*scid*	116.5±13	291.2±44	256.0±41	268.2±46.2
NOD	86.0±3.8	425.0±47*	483.3±86.2*	355.2±208.8

Basal blood glucose and insulin levels were measured as it was described in Materials and methods. Data are expressed as the mean±S.E.M. Statistical differences against age-matched control C57BL/6 mice age-matched, * $P<0.05$; ** $P<0.002$. (One-way ANOVA followed by Bonferroni t-test).

regardless of the age during the prediabetic period, and the difference was significant at 2 and 8 weeks of age ($P<0.05$ and 0.002, respectively). NOD*scid* also had significant lower glycemia than C57BL/6 mice at 4 and 8 weeks of age ($P<0.05$ in both cases). At 16 weeks of age, blood glucose levels were similar in C57BL/6, nondiabetic NOD (i.e. those which were not yet or will never become diabetic) and NOD*scid* mice, essentially because in control C57BL/6 mice glycemia decreased significantly between 8 and 16 weeks of age ($P=0.006$). In 16-week-old NOD mice which were already diabetic for at least 3 weeks, mean glycemia values were 26.4±3.8 mmol/l. All C57BL/6 mice, which were treated with STZ at 12 weeks of age, were positive for urinary glucose 48 h after drug injection. One month after, i.e. at 16 weeks of age, STZ-treated mice showed marked hyperglycemia (15.8±2.0 mmol/l).

Concerning basal nonfasting insulinemia (Table 1), NOD mice had significantly higher levels than controls at 4 and 8 weeks of age ($P<0.005$ for the two ages). NOD*scid* mice appeared to have higher insulin levels than controls but this effect did not reach significance. As expected in the group of nondiabetic 16-week-old NOD mice, due to the progressive autoimmune β-cell destruction and -exhaustion, mean insulin levels decreased but a strong variability still persisted from one animal from another. By contrast, insulinemia was stable in lymphocyte-deficient NOD*scid* mice, in which there was no β-cell destruction. Insulinemia was not measured in diabetic mice. It is known from the literature that blood insulin levels in diabetic NOD adult mice were low in consonance with undetectable islet cell insulin immunolabeling [2,3,5,25,29].

Importantly, data from Table 1 showed that mice with the NOD genetic background were characterized by a relative hyperinsulinemia and corresponding low glucose levels compared to control mice during early adult life and that, later on, this characteristic disappeared in NOD mice while it persisted in NOD*scid* mice.

3.2. Morphological characteristics of astrocytes

The immunohistochemical features of astrocytes present in the various groups of 16-week-old mice are given in Fig. 1A–F. Fig. 1A–C compared GFAP immunostaining at low magnification in C57BL/6, STZ-treated and diabetic NOD mice. It can be seen that GFAP immunostaining of astrocytes was attenuated in the stratum radiatum of control C57BL/6 hippocampus (Fig. 1A), while increased $GFAP^+$ staining appeared in STZ and diabetic NOD mice hippocampus (Fig. 1B, C, respectively). At higher magnification (Fig. 1D–E), $GFAP^+$ astrocyte shape appear to differ, in that diabetic NOD astrocytes showed a more dendritic shape than STZ-treated C57BL/6 mice, which showed a more condensed staining of perikaryon (cf. Fig. 1D, E, respectively). Fig. 1F shows the intimate structural relationship in STZ diabetic mouse hippocampus between

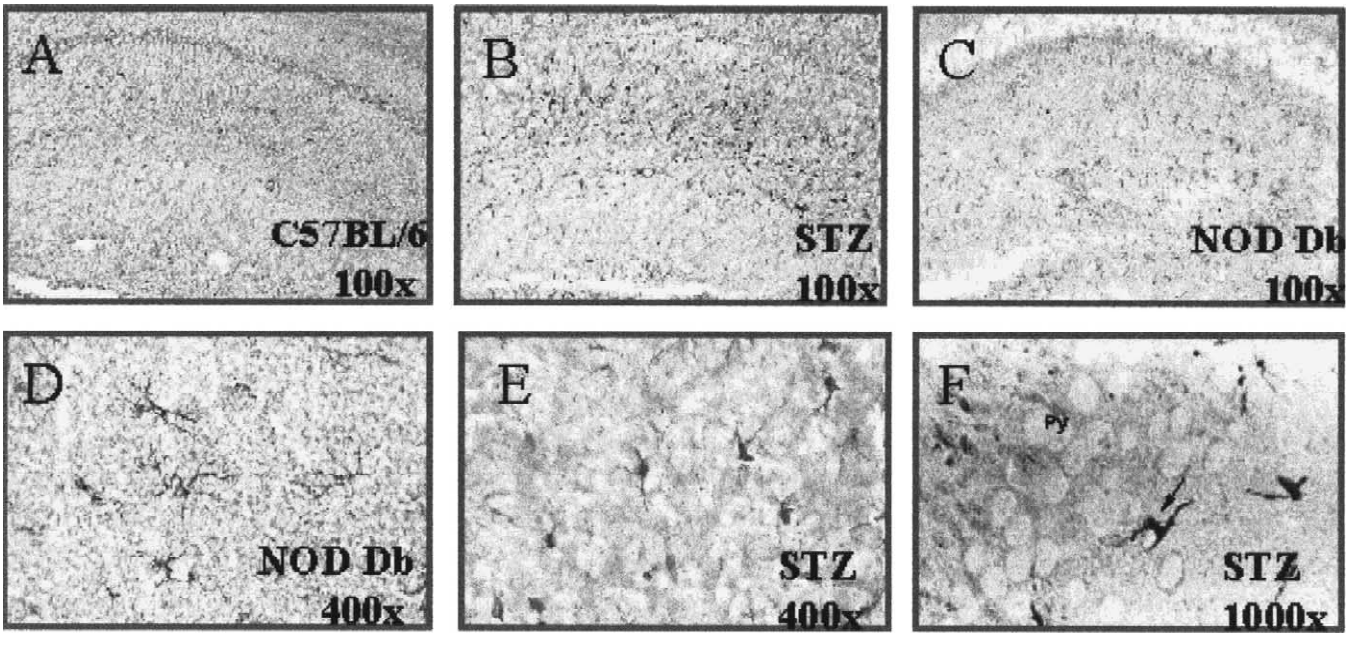

Fig. 1. (A–E), photomicrographs showing $GFAP^+$ cells in hippocampal stratum radiatum from 16-week-old female control C57BL/6 (A), STZ-treated (B) and diabetic NOD mice (C) observed at low magnification (100×). Note the attenuated $GFAP^+$ cells in C57BL/6 controls (A), numerous cells in STZ-diabetic animals (B) and also diabetic NOD mice (C). D and E shows the characteristics of $GFAP^+$ astrocytes at higher magnification (400×) astrocytes in diabetic NOD (D) and STZ-treated C57BL/6 (E) mice. Note the dendritic shape of $GFAP^+$ cells in diabetic NOD and more condensed forms in STZ-treated mice. F: Unstained pyramidal neurons of CA1 area (Py) and $GFAP^+$ cells (arrows) are intimately connected in the STZ-treated mice (1000×).

$GFAP^+$ cells and unlabelled neurons, as indicated with the arrow.

According to the existence of these morphological differences among the various groups of mice, we quantified the number of $GFAP^+$ cells and their cellular area.

3.3. Hippocampal astrogliosis is an early characteristic feature of the NOD genetic background in the absence of diabetes

The number and area of $GFAP^+$ astrocytes in the NOD stratum radiatum hippocampal region are shown in the upper and lower panel of Fig. 2. When considering NOD, NOD*scid* and C57BL/6 mice at 2, 4 and 8 weeks of age, i.e. long before clinical onset of diabetes, a remarkable difference already exists in the number of $GFAP^+$ astrocytes among mice with the NOD genetic background and the control C57BL/6 strain (Fig. 2, upper panel). In 4- and 8-week-old NOD and NOD*scid* mice but not in 2-week-old animals of both strains, the number of $GFAP^+$ cells was significantly higher than in controls (Fig. 2, upper panel; $P<0.01$ for both groups of mice at 4 weeks of age, and $P<0.05$ at 8 weeks of age, vs. age-matched controls). By contrast, in 2-, 4- and 8-week-old mice the cell area of $GFAP^+$ astrocytes did not show significant differences among the various groups (Fig. 2, lower panel).

3.4. Hippocampal astrogliosis is worsened in older mice with the NOD genetic background and at clinical onset of diabetes

As also shown in the upper panel of Fig. 1, $GFAP^+$ number was increased in 16-week-old NOD*scid*, nondiabetic NOD and diabetic NOD mice compared with age-matched controls ($P<0.05$ for the first group and $P<0.01$ for nondiabetic and diabetic NOD, respectively). Moreover, the number of $GFAP^+$ cells was higher in diabetic NOD mice than nondiabetic NOD and NOD*scid*. In addition, as shown in the lower panel of Fig. 2, at 16 weeks of age, the GFAP immunoreactive cell area was significantly increased in 16-week-old NOD*scid*, nondiabetic and diabetic NOD mice compared to controls ($P<0.01$ for the two first groups and $P<0.001$ for the last one). The cell area positive for GFAP was significantly higher in diabetic NOD mice than in the three other groups: nondiabetic NOD ($P<0.05$), NOD*scid* ($P<0.05$) and control mice ($P<0.001$).

3.5. Hippocampal astrogliosis is also present in STZ-treated diabetic mice

As shown in Fig. 3, the number of $GFAP^+$ astrocytes increased 3-fold in the hippocampal stratum radiatum of the STZ-diabetic female mice compared with age-matched vehicle-treated nondiabetic controls ($P<0.005$). As observed in the hippocampus of diabetic NOD mice, GFAP immunoreactive cell area was also significantly increased in the STZ-diabetic group ($P<0.05$, Fig. 3).

4. Discussion

The present data showed that a change in GFAP immunoreactivity is a common denominator of the spontaneous model of T1D, the NOD mouse and the pharmacological STZ-treated mouse model. However, the study of the kinetics of events in the NOD mouse and the lymphocyte-deficient NOD*scid* strain highlighted the early appearance of hippocampal astrocyte reaction in possible relationship with a period of hyperinsulinemia and corresponding low glucose levels. Moreover, hyperglycemia per se had, in both models of T1D, a powerful stimulatory effect on hippocampal astrocyte changes. Quantitatively, however, it is possible that astrocyte reaction found in both models of diabetes was moderate, in comparison with the strong astrogliosis developing after CNS injury, which compromises neurons and evokes an inflammatory response [34,45].

Examination of the number of $GFAP^+$ cells and the GFAP-immunoreative cell area in mice with the NOD genetic background, demonstrated that the first parameter changing from 4 weeks of age, was the $GFAP^+$ cell number. Later on, in 16-week-old NOD-related strains, regardless of the absence of presence of diabetes, the increased $GFAP^+$ cell number was accompanied by increased GFAP immunoreactive cell area, which may indicate astrocyte hypertrophy. As a possible explanation, we suggest that paradoxical hyperinsulinemia and corresponding low glycemia levels that were observed during early adult life in both NOD and NOD*scid* mice and persisted in old NOD*scid* mice, might play a role in this phenomenon [3,26,55]. Insulin may have a direct effect on astrocytes, since insulin receptors were found on astrocytes and insulin was shown to simulate Glut1 mRNA and glucose uptake in astroglial cells of the brain [53,54]. Moreover, neonatal hyperinsulinemia in rats induced astrocytosis in other brain regions, such as the hypothalamus [50,51]. It should also be noted that, in diabetic humans and animals, iatrogenic insulin-related hypoglycemia causes cognitive deficits and selective damage to the cerebral cortex and hippocampus, with evidence of necrosis, cell shrinkage, neuronal edema with vacuolation and nuclear condensation [17,23,70]. Therefore, in mice with the NOD genetic background hyperinsulinemia per se and/or its lowering effect on glycemia might affect hippocampal astrocytes and perhaps also neurons.

Both parameters reflecting astrocyte reactivity, number and cell area, were further exacerbated when NOD mice became diabetic. The intensification of astrocyte reaction in diabetic NOD mice suggests an additional role of hyperglycemia after the transient period of hyperin-

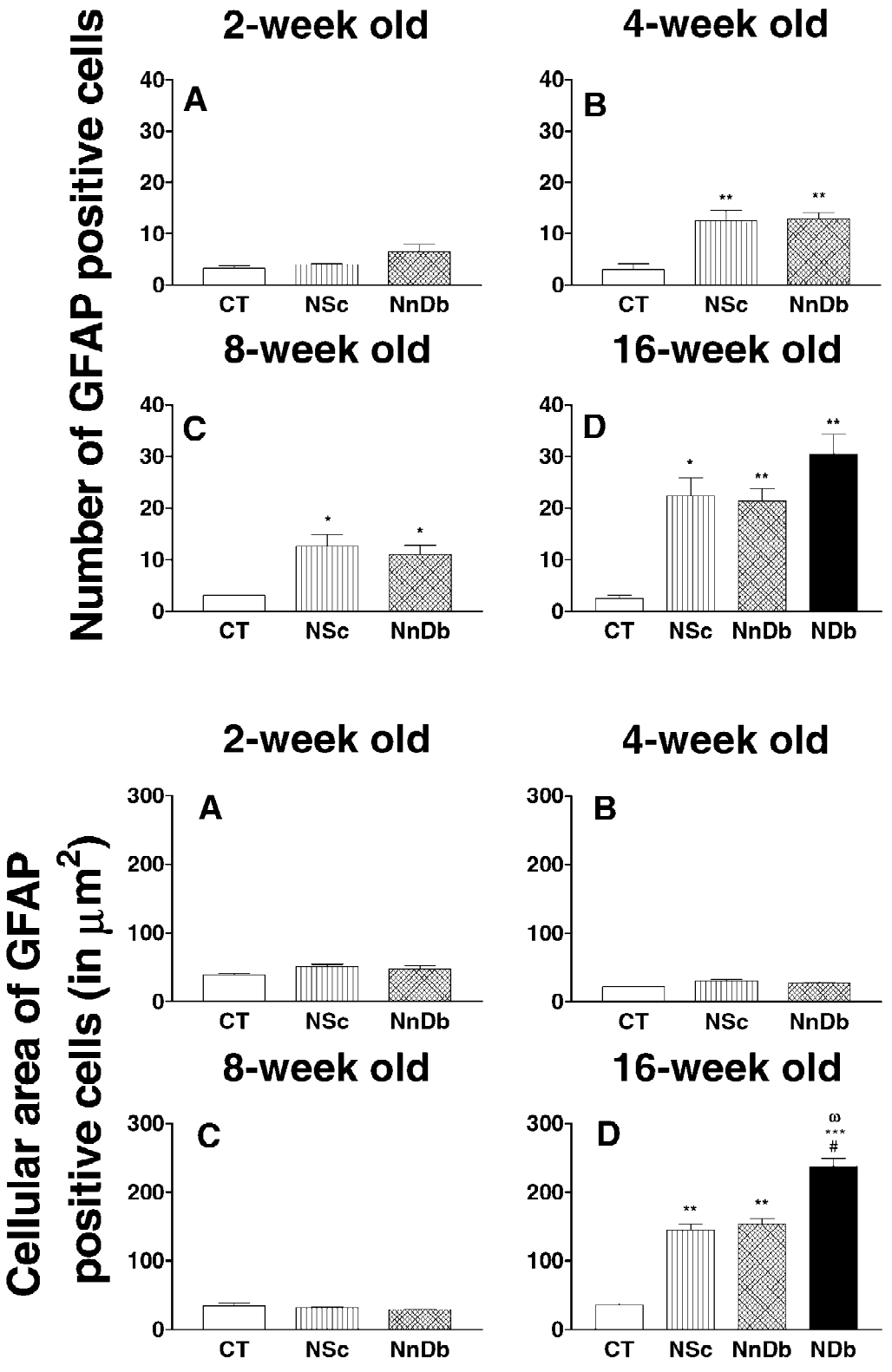

Fig. 2. Upper panel: Quantitation by computerized image analysis of the number of $GFAP^+$ cells (per area of 65×10^3 μm^2) in hippocampal stratum radiatum region from control C57BL/6 (CT), lymphocyte-deficient NOD*scid* (NSc) and nondiabetic (NnDb) and diabetic (NDb) NOD females of 2- (A), 4- (B), 8- (C) and 16 weeks of age (D). Note the increased astrocyte number in the strains with the NOD genetic background from 4 weeks of age and the aggravating effect of diabetes in NOD mice, * $P<0.05$ and ** $P<0.01$ vs. C57BL/6; (one-way ANOVA followed by Bonferroni post-hoc test). Lower panel: Quantitation by computerized image analysis of the cellular area of $GFAP^+$ cells in hippocampal stratum radiatum region from control C57BL/6, lymphocyte-deficient NOD*scid* (NSc) and nondiabetic (NnDb) and diabetic (NDb) NOD females of 2- (A), 4- (B), 8- (C) and 16 weeks of age (D). Note the significant increase of cellular area only in 16-week-old mice with the NOD genetic background in the absence or presence of diabetes, ** $P<0.01$, *** $P<0.001$ vs. C57BL/6, #<0.05 P vs. NOD*scid* mice, ∞<0.05 vs. P NOD nondiabetic mice (one-way ANOVA followed by Bonferroni post-hoc test).

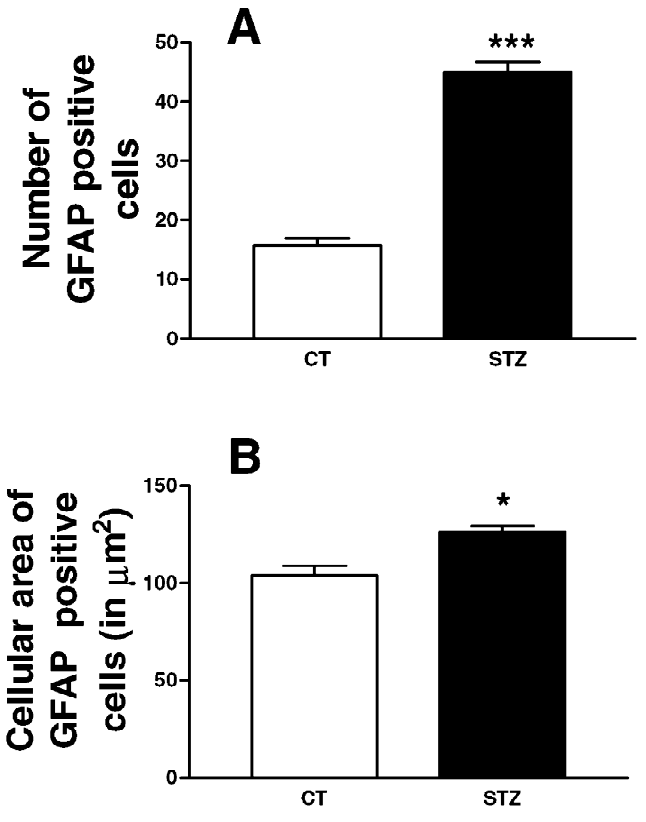

Fig. 3. Quantitation, using computerized image analysis, of the number (A) and cellular area (B) of $GFAP^+$ cells in hippocampal stratum radiatum region (per area of 65×10^3 μm^2) of 16-week-old vehicle-treated nondiabetic (CT) and STZ-treated diabetic C57BL/6 females (STZ, 200 mg/kg given i.p. 1 month before sacrifice). Both the number of $GFAP^+$ cells and the cellular area of $GFAP^+$ cells are significantly increased in diabetic animals. *** $P<0.005$, * $P<0.05$ vs. vehicle-treated C57BL/6 (one-way ANOVA followed by Bonferroni post-hoc test).

sulinemia and corresponding low glycemia. Hyperglycemia was also probably the leading mechanism for increased astrocyte reactivity in the STZ-induced model. Long standing hyperglycemia in STZ-diabetic rats has also been associated with diffuse cerebral astrogliosis [42], abnormal neurogenesis in the dentate gyrus [28], damage of pyramidal cells of the hippocampus [37] and neuronal apoptosis [58]. The last change also occurred after infusion of high glucose solutions, pointing to a neurotoxic role of hyperglycemia ascribed to increased oxidative stress [59]. Toxic effects of hyperglycemia in the brain derived from increases in the polyol pathway, protein glycation, oxidative stress and disturbed calcium homeostasis [20].

In both models studied, increased $GFAP^+$ cell number and immunoreactive area were observed in the white matter (stratum radiatum) of a highly vulnerable CNS region, such as the hippocampus. It is possible that several and not a single mechanism may account for the astrocyte reaction in the genetic and drug-induced models of diabetes. These would include cell migration from distant sites to the hippocampus, changes in cell reactivity from a $GFAP^-$ to a $GFAP^+$ phenotype and also astrocyte proliferation. According to some authors [45,64], astrocyte hypertrophy is the dominant element in reactive gliosis, whereas a small number of cells proliferate. It is yet unproven whether hippocampal astrocytosis in our animal models followed this general pattern. Coexistence of increased number of $GFAP^+$ astrocytes and cell hypertrophy in animals with overt hyperglycemia makes difficult to differentiate which event comes first. However, it should be noted that in NOD-related strains studied under the early low glycemic conditions, increased number of $GFAP^+$ cells preceded changes in immunoreactive cell area.

Both cycling between hypoglycemia and hyperglycemia constitute powerful stressors to the living animal. In this regard, it has been demonstrated that NOD mice during the prediabetic and diabetic stages, STZ-treated rats and the type II diabetic ob/ob mouse, showed hyperresponse to stress [63]. Hypoglycemia also brings an exaggerated hypothalamic–hypophysial axis response [2–4,10,16,26]. The neurochemical changes brought in motion by hyperglycemic or hypoglycemic stress could induce in part the astrocyte reaction reported in the present investigation. Therefore, astrocyte abnormalities found in NOD mice and STZ-treated animals might represent an adaptive response to changing blood glucose levels in order to provide neuroprotection. A beneficial role for astrocytes was supported by data illustrating that reactive, GFAP-expressing astrocytes bring neuroprotection during metabolic insults, stress or injury by secretion of growth factors, substrate-bound neurite promoting factors and removal of neurotoxins and excess glutamate [30,31,33]. Astrocytes may also protect neurons by increasing glucose uptake, metabolism and transport [38,68], in addition to being necessary for preservation of myelin and normal white matter architecture [34]. Moreover, the close connection of astrocytes with the blood–brain barrier make them very early sensors of variations of glucose homeostasis, that they can communicate to the neurons [38]. Finally, it is worth noting that in the STZ model, at the time of overt diabetes, some neuronal changes appeared [61]. In this work, we found in pyramidal hippocampal cells and granule cells of dentate gyrus of STZ-diabetic mice, increased Fos positive nuclei and increased NADPH-diaphorase (nitric oxide synthase) histochemical staining, suggesting disturbances in neuronal function [61].

To conclude, these data and data from others [8,20,39,40,42] show that diabetes has a substantial impact on the brain. The observed changes in T1D might result from successive periods of low glycemia and hyperglycemia in NOD mice, whereas it is a main response to hyperglycemia in STZ-treated mice. We would like to suggest that increases in GFAP immunoreactivity represented an adaptive process, since adaptive plasticity is a well known phenomenon in the hippocampus [20,31,32,53,54]. Finally, in terms of astrocyte changes,

the CNS impact of diabetes present strong similarities with stress and aging.

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