Journal of Neuroendocrinology, 2004, Vol. 16, 704–710 doi:10.1111/j.1365-2826.2004.01223.x

Oestradiol Restores Cell Proliferation in Dentate Gyrus and Subventricular Zone of Streptozotocin-Diabetic Mice

F. Saravia,*† Y. Revsin,* V. Lux-Lantos,‡ J. Beauquis,* F. Homo-Delarche§ and A. F. De Nicola*†
*Laboratory of Neuroendocrine Biochemistry, Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina.
†Department of Biochemistry, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina.
‡Laboratory of Neuroendocrinology, Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina.
§CNRS UMR 7059, Université Paris 7/D, Diderot, Paris, France.

Key words: type 1 diabetes, oestrogens, hippocampus, adult neurogenesis, bromodeoxyuridine.

Abstract

Type 1 diabetes mellitus correlates with several brain disturbances, including hypersensitivity to stress, cognitive impairment, increased risk of stroke and dementia. Within the central nervous system, the hippocampus is considered a special target for alterations associated with diabetes. Neurogenesis is a plastic event restricted to few adult brain areas: the subgranular zone of the dentate gyrus and the subventricular zone (SVZ). First, we studied the ability for neurogenesis in the dentate gyrus and SVZ of chronic diabetic mice induced by streptozotocin (STZ). Using bromodeoxyuridine (BrdU) labelling of cells in the S-phase, we observed a strong reduction in cell proliferation rate in both brain regions of diabetic mice killed 20 days after STZ administration. Second, because oestrogens are active neuroprotective agents, we investigated whether 17β-oestradiol (200 μg pellet implant in cholesterol during 10 days) restored brain cell proliferation in the diabetic mouse brain. Our results demonstrated a complete reversibility of dentate gyrus cell proliferation in oestrogen-treated diabetic mice. This plasticity change was not exclusive to the hippocampus because oestrogen treatment restored BrdU incorporation into newborn cells of the SVZ region of diabetic animals. Oestrogen treatment did not alter the hyperglycemic status of STZ-diabetic mice. Moreover, oestrogen did not modify BrdU incorporation in control animals. These data show that oestrogen treatment strongly stimulates brain neurogenesis of diabetic mice and open up new venues for understanding the potential neuroprotective role of steroid hormones in diabetic encephalopathy.

Introduction

The various behavioural, neuroendocrine and neurophysiological abnormalities appearing in uncontrolled type 1 diabetes mellitus support the concept of a diabetic encephalopathy (1–5). At the cellular level, diabetes can alter, among other structures, hippocampal glial cells and neurones. Astrogliosis, with increased expression of the glial fibrillary acidic protein (GFAP) is a prominent hippocampal feature in spontaneous and induced models of type 1 diabetes mellitus (6, 7), such as the nonobese diabetic mice, and in streptozotocin (STZ) diabetic rat, respectively. The reactive astrocytes found in diabetic mice may provide neuroprotection to the ailing neurones, in agreement with the role of astrocytes in trauma, ageing and degenerative diseases (8, 9).

In type 1 diabetes mellitus, hippocampal neurones are indeed highly vulnerable (10). Dendritic atrophy, down-regulation of glucocorticoid receptors, altered expression of insulin-growth factor-I (IGF-I) receptors, decreased glucose transporters and susceptibility to apoptosis are described in the hippocampus of diabetic animals (5, 7, 10–14). Moreover, STZ-treated mice present increased expression of early genes in the pyramidal cell layer and dentate gyrus, and hyperactivity of NADPH-diaphorase/nitric oxide synthase in the CA3 subfield (15). In animals with type 1 diabetes mellitus, the increased production of nitric oxide, together with the beneficial effects of antioxidants, suggests that oxidative stress may be involved in neuronal pathology (16, 17).

Physiologically, it is worth noting that neurogenesis continues throughout adulthood in dentate gyrus and ventricular subependyma around the hippocampus known as the 'subventricular zone' (SVZ) (18). In these regions, cell proliferation is usually assessed by measuring incorporation of the thymidine analogue, bromodeoxyuridine (BrdU), which is

Correspondence to: Dr Alejandro F. De Nicola, Instituto de Biologia y Medicina Experimental, Obligado 2490, 1428 Buenos Aires, Argentina (e-mail: denicola@dna.uba.ar).

taken up by dividing cells during the S-phase. This technique has shown that neural progenitors in the dentate gyrus proliferate, migrate into the granular layer and differentiate into granule cells (19), whereas SVZ-born neurones are destined for the olfactory bulb (20). This neuronal cell proliferation shows a marked plasticity in response to hormones and environmental factors (20, 21). Functionally, neurogenesis in the dentate gyrus is associated with the formation of some types of hippocampal-dependent memories (22) and the depletion of proliferating cells correlates with impaired acquisition of a fear-conditioned response (23). For example, in diabetic animals, 48 h after diabetes induction, a pronounced reduction of cell proliferation exists in the dentate gyrus subgranular zone (24) in association with changes in learning and memory, again suggesting hippocampal dysfunction (1–3, 14, 25, 26).

In the central nervous system (CNS), oestrogens exert trophic and protective effects under normal and pathological conditions (27–30). Recently, it has been established that oestrogens can be synthesized by hippocampal neurones in adult rats (31). Regarding the control of neurogenesis, oestrogens increase the number of immature neurones of the dentate gyrus (32) and prevent neurotoxin-induced granule cell apoptosis (33). These protective effects may consequently modify behaviours including learning and memory (22). The oestrogen effect may be only transient because granule cell proliferation is no longer stimulated after prolonged exposure to high oestrogen doses (34). In agreement with the stimulatory effect in the dentate gyrus, oestrogens also modulate proliferation and differentiation of cells cultured from wall lining of the SVZ of adult rats (35). In molecular terms, oestrogen action in the dentate gyrus and SVZ may be mediated by the oestrogen receptor (ER) because one or both isoforms of ERα and β are found in stem cells of the ventricular wall of adult rat (35) while the mRNAs for both ERα and ERβ and ERα immunoreactivity coexist in the infragranular layer and hilus of the dentate gyrus (28, 36). The role of the intracellular receptor in the dentate gyrus is supported by an ER antagonist blocking the induction of neurogenesis induced by IGF-I (37). In addition, oestrogens also influence second messengers and different kinases in the CNS by pathways independent of the classical ER (28), suggesting a multifactorial modulation of cell proliferation in dentate gyrus and SVZ.

In the present study, we investigated whether the reduction of granule cell proliferation reported in the dentate gyrus in type 1 diabetes mellitus (24) is reflected by a definitive long-term damage or a reversible condition. We resorted to oestradiol treatment of STZ-diabetic and control mice, considering the reported stimulation of cell proliferation and presence of ER in areas related to neurogenesis of adult animals. The results demonstrated: (i) normalization of dentate gyrus cell proliferation in oestrogen-treated diabetic mice; (ii) that this plasticity change was not exclusive to the hippocampus because the reduced BrdU incorporation into SVZ structures of diabetics was also restored by oestrogen treatment; and (iii) that oestrogen effects on cell proliferation occurred without changes in the hyperglycemic status of STZ-diabetic mice.

Materials and methods

Animals and treatment

Male C57BL/6 mice were housed under conditions of controlled humidity and temperature (22 °C), and a 12 : 12 h light/dark cycle (lights on 07.00 h) at the facility of the Institute of Biology and Experimental Medicine (Buenos Aires, Argentina). Experimental procedures followed the NIH Guide for the Care and Use of Laboratory Animals (Assurance Certificate #A5072-01). Twelve-week-old mice (weighing approximately 30 g) received a single i.p. dose of 200 mg kg body weight STZ (Sigma, St Louis, MO, USA) dissolved in 0.5 M sodium citrate buffer or the vehicle alone (n = 6–7 animals per group). Two days after injection, glycosuria was determined using Keto-Diastix (Bayer Diagnostics, Buenos Aires, 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 colourimetry (Accutrend GC, Boehringer Mannheim, Mannheim, Germany). Animals with glycaemia higher than 11 mmol/l glucose were classified as overtly diabetic. STZ-treated mice showed marked hyperglycaemia (15.8 ± 2.0 mM) 48 h after STZ injection. As shown in Fig. 1, 10 days after STZ or vehicle injection, a 12 mg cholesterol pellet containing 200 μg of 17β-oestradiol (Sigma), or only cholesterol, was placed s.c. under light ether anaesthesia to selected groups of diabetic or control mice. Finally, 10 days after oestradiol or vehicle treatment, animals were weighed and decapitated. Their blood and tissues were collected at the time of killing (12.00 h) for determination of glycaemia and weight of the testis and hypophysis. As determined by radioimmunoassay (38), the oestradiol pellet implant in mice produced highly elevated levels of circulating oestradiol (497.3 ± 70.4 pg/ml) compared to normal male mice (14.93 ± 2.31 pg/ml).

BrdU administration and immunocytochemistry

Mice received a single i.p. injection of 5-bromo-2′-deoxyuridine (BrdU) (Sigma) at 50 μg/g body weight (10 mg/ml stock, dissolved in 0.9% saline) and were killed 2 h later. At this time period, BrdU incorporation measures the extent of cell proliferation only (39). Before perfusion, mice were deeply anaesthetized by i.p. injection of ketamine (33.3 mg/100 g body weight). They were perfused transcardially with 30 ml of 0.9% saline followed by 50 ml 3% paraformaldehyde (PFA) in phosphate buffer (PB), pH 7.4. Brains were incubated overnight in 3% PFA. On the next day, they were transferred to Tris-buffered saline (TBS), pH 7.4 and processed for free-floating BrdU immunocytochemistry. Brains were sectioned frontally at 50 μm using a vibrating microtome.

For DNA denaturation and BrdU detection, sections from each mouse were processed separately. They were incubated in prewarmed 50% formamide/2 × SSC at 65 °C for 10 min, rinsed in 2 × SSC for 10 min, incubated in 2 N HCl at 37 °C for 30 min, rinsed in 0.1 M boric acid, pH 8.5, for 10 min, washed three times in TBS, pH 7.4 and blocked for 30 min in TBS with 0.1% Triton X-100 and 10% horse serum. Sections were incubated for 48 h at 4 °C in a shaker with rat anti BrdU mAb (1 : 200, Accurate Chemicals, Westbury, NY, USA) diluted in blocking solution. After three washes in 0.1% Triton

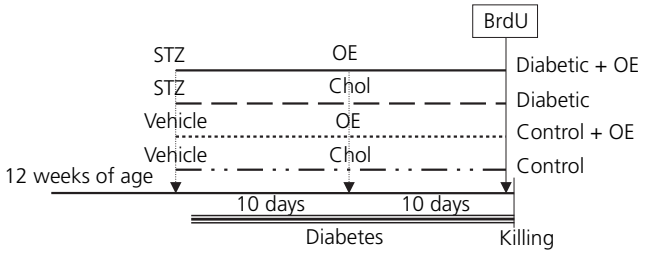

FIG. 1. Summary of the experimental design. C57BL/6 males of 12 weeks of age were injected with streptozotocin (STZ) (200 mg/kg) or vehicle (citrate buffer). Ten days afterwards, animals were s.c. implanted with a cholesterol pellet containing 200 μg of 17β-oestradiol (OE) or vehicle alone (chol). After 10 days, mice were injected with a single dose of 5-bromo-2 deoxyuridine (BrdU) (50 mg/kg) and killed 2 h later. This procedure originated the following four groups (from top to bottom): diabetic + OE, diabetic, control + OE and control, respectively.

X-100-TBS, sections were incubated with the secondary antibody, a biotinylated anti-rat IgG (1 : 200, Sigma) in 0.1% Triton X-100-TBS during 2 h in a shaker at room temperature. After three washes in TBS, they were processed following the ABC kit instructions (Vector Laboratories, CA, USA). For development, we used 3,3′-diaminobenzidine (DAB) at 0.5 mg/ml, 0.05% H_2O_2 at room temperature. Sections were mounted in gelatin-coated glass slides and air dried. After counterstaining with cresyl violet to better identification of the brain region of interest, the slides were dehydrated with graded ethanols and xylene and mounted with Permount (Fisher Chemical, Fairlawn, NJ, USA). Nonspecific staining was assessed in the absence of primary antibody.

Quantitative analysis of BrdU positive cells

For each group, 9–10 sections from each animal were analysed. The level of the dentate gyrus for counting BrdU labelled cells corresponded to the 'middle dentate gyrus', in which the suprapyramidal and the infrapyramidal blades are joined at the crest region and the dentate gyrus is orientated horizontally beneath the corpus callosum (40). In sections containing the 'middle' dentate gyrus, BrdU positive cells were also counted in the entire SVZ. The SVZ comprised the wall of the posterior lateral ventricle near the fimbria hippocampus and the hippocampal arch above the CA1–CA2 subfields just below the corpus callosum (20). Localization of dentate gyrus and SVZ corresponded to Plate(s) 16–17, A 2, 400–2500 from the stereotaxic atlas of the mouse brain (41).

Cell counting in the hippocampal dentate gyrus and SVZ was performed by computerized image analysis using Optimas II software coupled to an Olympus BH-2 microscope (Melville, NY, USA) equipped with a VT-C330N video camera (6). Data were presented as the mean ± SE BrdU-positive cells corresponding to control plus vehicle (cholesterol) (n = 6 animals), control plus oestrogen (n = 6), diabetic plus vehicle (n = 5), and diabetic plus oestrogen (n = 5) groups. Manual counting of BrdU positive cells was also performed at ×400 magnification and no differences were found with the software-generated counting. The investigators who processed the cell counting were blinded to the procedure.

Double immunofluorescence and confocal microscopy

The neuronal or glial phenotype of newborn cells was studied using double immunofluorescence. Briefly, sections were processed as described above and incubated with rat anti BrdU (1 : 200) and one of the following specific antibodies: mouse anti-β-III tubulin (1 : 1000 Promega, Madison, WI, USA), anti-Neu-N (1 : 100, Chemicon, Temecula, CA, USA) or rabbit anti-GFAP (1 : 400 Sigma). After washing in buffer, sections were incubated for 2 h at RT with the secondary antibodies (1 : 200). BrdU was detected with goat-anti-rat IgG coupled to FITC, β-III tubulin with horse-anti mouse IgG coupled to rhodamine and GFAP with horse antirabbit IgG coupled to rhodamine. After sequential washes, sections were mounted on gelatin-coated slides and examined under a Nikon Eclipse E 800 confocal scanning laser microscope. Images were acquired sequentially in a line-scanning mode through an optical section of 1 μm in the z-axis, and merged using Nikon EZC1 version 2.1 software (Nikon, Melville, NY, USA).

Statistical analysis

Group differences for cell counting, blood glucose levels, and body and tissue weight were determined by one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. $P < 0.05$ was considered statistically significant.

Results

Glycaemia, body and tissue weight

Morning blood glucose levels of diabetic mice measured 20 days from the time of diabetes induction were slightly lower in oestrogen-treated animals (601 ± 24.4 mg/dl) compared to untreated mice (654 ± 76.4 mg/dl), although differences did not reach significance. Body and tissue weight was recorded to ascertain the clinical effectiveness of oestrogen treatment lasting for 10 days in the diabetic group. In the beginning of the experiments, the mean of body weight was 30.52 ± 1.02 g, while at the time of killing (i.e. 20 days after diabetes induction), the measure was as follows: control mice implanted with cholesterol: 33.35 ± 0.98; control with oestrogen: 31.86 ± 1.48; diabetic with cholesterol: 23.1 ± 1.1 ($P < 0.001$ versus control + cholesterol); and diabetic with oestrogen: 22.4 ± 0.7 ($P < 0.001$ versus control + oestrogen). Testis weight was significantly reduced by oestrogen treatment in the diabetic group (80.7 ± 3.3 mg) compared to the control (102.2 ± 7.5 mg, $P < 0.05$) and diabetic + vehicle groups (88.7 ± 2.9 mg). Pituitary weight was subnormal in vehicle-receiving diabetic mice (1.2 ± 0.07 mg, $P < 0.05$), compared to control mice (2.05 ± 0.1 mg) and oestrogenized diabetics (1.9 ± 0.07 mg). Thus, the deleterious effect of uncontrolled diabetes on pituitary weight was recovered by oestrogen treatment of diabetic mice. As expected, oestrogen-treated animals showed testicular atrophy.

BdrU incorporation into dentate gyrus and SVZ cells.

In control mice, several BrdU labelled cells were observed in the suprapyramidal and infrapyramidal layers at the middle level of the dentate gyrus (Fig. 2A). Cell counting in both layers of each dentate gyrus demonstrated a mean of 14 ± 2 labelled cells in control mice, a figure not significantly modified 10 days after oestrogen treatment (Fig. 2A, Ctl versus Ctl + OE; Fig. 3A). By contrast, a marked reduction to 4.8 ± 1.9 labelled cells was found in the steroid-naïve diabetic group ($P < 0.01$ versus control) (Fig. 2A, STZ; Fig. 3A), which was restored to control levels after 10 days of oestrogen treatment (14.9 ± 1.1; $P < 0.001$ versus diabetic + vehicle), as shown in Fig. 2(A) (STZ + OE) and in quantitative form in Fig. 3(A). At higher magnification (Fig. 2, inserts a–c), BrdU-labelled cells from vehicle-treated controls, controls plus oestrogen and oestrogenized diabetic mice, respectively, showed similar clusters of dark stained nucleus and irregular shape, without morphological differences between untreated and oestrogenized groups. The pattern of BrdU staining is representative of immature cells under division as previously reported in the literature (42).

BrdU-labelled cells were also observed in areas of the SVZ selected for counting (Fig. 2B). These areas enclosed the wall of the lateral ventricle near the fimbria of the hippocampus and the area below the corpus callosum and above the CA1 and CA2 subfields. Representative photomicrographs of BrdU positive cells in the SVZ in control (Ctl), control plus oestrogen (Ctl + OE), diabetic (STZ), and diabetic plus oestrogen (STZ + OE) groups are shown in Fig. 2(B). The number of BrdU positive cells in SVZ from control, control plus oestrogen, and diabetic plus oestrogen groups was similar as shown histologically (Fig. 2B, Ctl, Ctl + OE and STZ + OE, respectively) and quantitatively (Fig. 3B). By contrast, diabetic mice implanted with the cholesterol vehicle pellet presented a significant reduction in cells incorporating the thymidine analogue ($P < 0.01$ versus all other groups) (Fig. 2B, STZ and Fig. 3B). Thus, a clear enhancement of BrdU incorporation was caused by oestradiol treatment of diabetic mice.

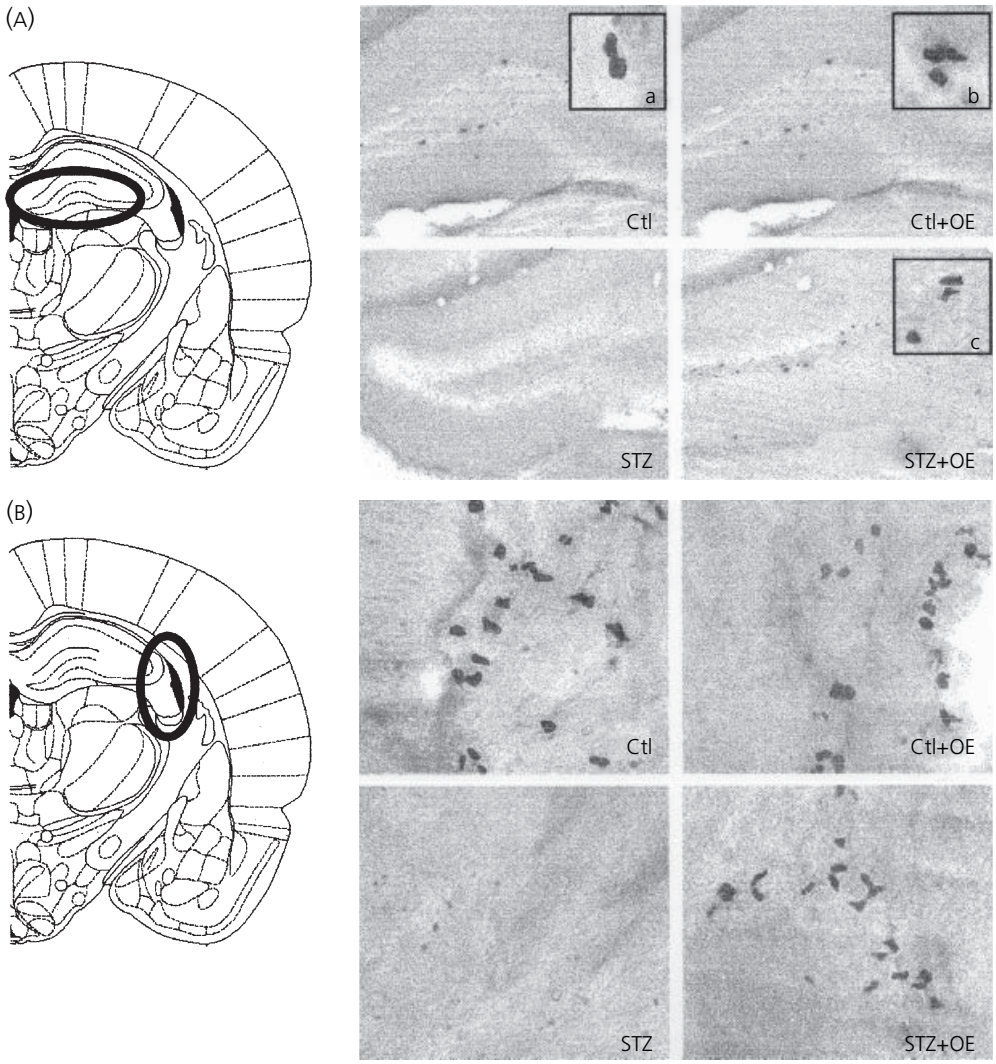

FIG. 2. (A) Representative photomicrographs of 5-bromo-2 deoxyuridine (BrdU)-immunopositive cells in the dentate gyrus (dentate gyrus). Cells incorporating BrdU were easily seen in vehicle-treated control (Ctl), oestrogen-treated control mice (Ctl + OE) and oestrogen-treated diabetic mice (STZ + OE). By contrast, they were practically absent in vehicle-treated diabetic mice (STZ). Magnification × 40. Photographs correspond to the suprapyramidal and the infrapyramidal blades encircled in black in the brain schema on the left. Higher magnification of BrdU-incorporating cells are shown in the inserts corresponding to Ctl (a), Ctl + OE (b) and STZ + OE (c) Magnification × 1000. (B) Representative photomicrographs of BrdU-immunopositive cells in the subventricular zone (SVZ). Group label as in the legend to Fig. 2(A). Abundant cells incorporating BrdU are seen in Ctl, Ctl + OE and STZ + OE, by contrast to the paucity of cells in STZ. As in (A), the brain region of interest is encircled in black. Magnification × 400.

The phenotype of BrdU-positive cells was studied using markers for neurones (β-III-tubulin and Neu-N) and astrocytes (GFAP). Confocal analysis showed that some cells in the dentate gyrus showed BrdU and β-III-tubulin colocalization. By contrast, BrdU-positive cells did not co localize with GFAP or Neu-N, a marker of more mature neurones (results not shown). Thus, in spite of the short time interval after BrdU administration, reaction with the β-III-tubulin antibody suggested some BrdU cells already expressed an immature neuronal phenotype.

Discussion

For the first time, the present study demonstrates the stimulatory effect of oestrogens on CNS cell proliferation in a pharmacological model of type 1 diabetes mellitus. STZ-induced diabetic male mice were overtly diabetic, showing a pronounced glycosuria and hyperglycaemia at the time of oestrogen pellet implantation (i.e. 10 days after STZ administration). After an additional 10 days of oestradiol treatment, changes of endocrine glands weight indicated the pharmacological effectiveness of the oestrogen treatment. Increased pituitary weight in oestradiol-implanted diabetic mice may result in concomitant lactotroph cell hyperplasia and angiogenesis (43). Testicular atrophy was also expected, due to oestrogenic inhibition of pituitary gonadotrophins and/or by a direct testicular action (44). The latter finding raises the question on whether androgen levels in the diabetic animals prevent neurogenesis. Although this possibility appears unlikely, it cannot be excluded by the current experimental design. Furthermore, the effect of oestradiol on neurogenesis cannot be due to amelioration of the diabetic

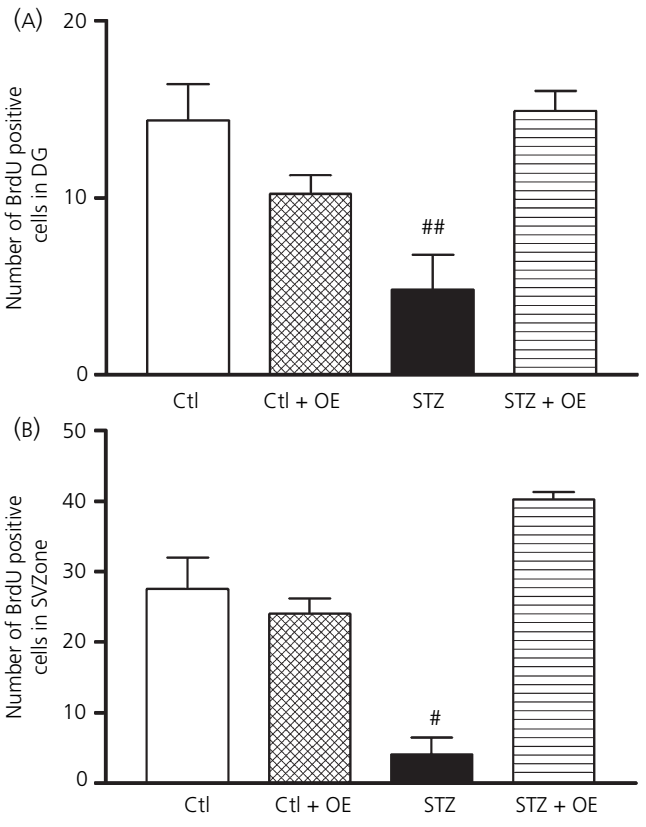

FIG. 3. Quantification of the number of 5-bromo-2 deoxyuridine (BrdU)-immunopositive cells in the (A) dentate gyrus (DG) and (B) SVZ of vehicle-implanted control mice (Ctl), oestrogen-treated control mice (Ctl + OE), vehicle-treated diabetic mice (STZ) and diabetic mice receiving oestrogen (STZ + OE). To study cell proliferation, animals were killed 2 h after administration of BrdU. Statistical significance was performed by ANOVA followed by Bonferroni's post-hoc test: (A) ##STZ versus all other groups [d.f. = 4, F(11,76), $P < 0.01$]. (B) #STZ versus all other groups [d.f. = 4, F(27,63), $P < 0.001$].

state because blood glucose values did not differ significantly between untreated or oestrogen-treated diabetic mice. In one study, oestrogen treatment did not significantly alter blood glucose and plasma insulin levels in STZ-diabetic rats but adversely affected body weight compared to controls (45).

The various alterations of neuronal structure, function and metabolism reported in type 1 diabetes mellitus (6, 7, 10–13, 15, 46) were recently shown to be accompanied by pronounced reduction of dentate gyrus cell proliferation (24), in agreement with our present data in the dentate gyrus. Additionally, we showed that BrdU incorporation, a measure of cell proliferation in animals killed 2 h after nucleotide administration (39), was diminished in SVZ cells of diabetic mouse brain. Regarding the fate of the proliferating cells, Seaberg and van der Kooy (20) stated that some cell types in the dentate gyrus generate neurones whereas others generate glial progeny, suggesting they are restricted progenitor cells. By contrast, these authors defined SVZ cells as stem cells because they were able to generate neurones, astrocytes and oligodendrocytes (20). Due to the schedule of BrdU administration performed in our experiments (i.e. one injection 2 h before sacrifice), only some proliferating cells showed colocalization with markers of immature neuronal phenotype like β-III-tubulin and we were unable to found Neu-N/BrdU or GFAP/BrdU positive cells. In a recent study, Kempermann *et al.* (47) emphasized that cell fate decisions towards neuronal development are made soon after division. Along this line, new experiments are in progress to identify the proliferating, migrating and differentiating cells after administration of several injections of BrdU to oestrogenized diabetic mice. In functional terms, the impaired neurogenesis in dentate gyrus may lie beneath the numerous deficits in learning and memory behaviour previously shown in diabetic animals (3, 26, 48). In agreement with this assumption, preliminary data from our group showed a better performance of diabetic mice treated with oestrogens in the elevated asymmetric plus-maze, a task related to explorative behaviour, compared to diabetic mice receiving vehicle.

Of particular interest, and shown here for the first time, oestradiol treatment during 10 days was able to completely restore cell proliferation in dentate gyrus and SVZ of diabetic mice. These data reinforce the notion that regions involved in neurogenesis are targets for oestrogens because ERα and ERβ were detected in the stem cells of the SVZ, whereas cells of the dentate gyrus expressed ERβ mRNA, retained radiolabel nuclear oestradiol injected into rats and contained ERα and ERβ immunoreactivity (28, 32, 49). It is also worth noting that oestradiol effects were exclusively observed in diabetic but not in normal mice. This finding was not entirely unexpected because it was reported that, in normal animals, oestrogens did not significantly affect the number of BrdU-immunoreactive cells in rat dentate gyrus unless a neurotoxin-induced reduction was provoked (32, 33). Furthermore, the possibility exists that the stimulation of cell proliferation following oestrogen treatment in diabetic mice follows from the growth-related effects of this hormone upon newly divided cells that differentiate into neurones in the hippocampus (50, 51). Mechanistically, it was established that oestrogens can activate cellular cascades involving growth factors, including IGF-I and its cognate receptor (37, 52). In normal rat hippocampus, IGF-I promotes proliferation and neuronal differentiation (53). In type 1 diabetes mellitus rat hippocampus, expression of IGF-I and its receptor are reduced and accompanied by apoptotic neuronal loss and functional cognitive impairment (14). Because oestrogens regulate IGF-I expression (52), it is not excluded that, in type 1 diabetes mellitus animals implanted with oestrogens, an oestradiol–IGF-I interaction takes place in SVZ and dentate gyrus cell proliferation, a hypothesis that deserves further analysis.

Finally, the literature reports that, in brain complications of diabetes mellitus, oestrogens also provided neuroprotection. Thus, oestradiol treatment of diabetic rats reduced infarct size of the striatum after transient middle cerebral artery occlusion (54), enhanced the subnormal brain glucose utilization rates of type 2 diabetic (db/db) mice (55), and improved the disturbances of cerebral energy metabolism and deterioration of memory functions of adult rats injected intracerebrally with STZ (48). Future studies are required to elucidate whether oestrogens can be therapeutically useful for normalization of neuronal disturbances and for improvement of learning disabilities of animals with type 1 diabetes mellitus and eventually diabetic patients.

Acknowledgements

The authors wish to thank Dr Alvarez Buylla and Bettina Seri for their kind advice regarding BrdU techniques and Dr Alejandro Schinder for the generous gift of β-III-tubulin antibody. The authors acknowledge the technical assistance of Analia Lima and Paulina Roig. This study was supported by an International agreement between CONICET-INSERM, and grants from the University of Buenos Aires (TM022), CONICET (PIP 02007). Y. Revsin was supported by a Doctoral Fellowship from the University of Buenos Aires.

Accepted 10 June 2004

References

1 Artola A, Kamal A, Ramakers GM, Gardoni F, DiLuca M, Biessels GJ, Cattabeni F, Gispen WH. Synaptic plasticity in the diabetic brain: advanced aging? *Prog Brain Res* 2002; **138:** 305–314.
2 Biessels GJ, van der Heide LP, Kamal A, Bleys RL, Gispen WH. Ageing and diabetes: implications for brain function. *Eur J Pharmacol* 2002; **441:** 1–14.
3 Gispen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. *TINS* 2000; **23:** 542–549.
4 Rowlands NE, Bellush LL. Diabetes mellitus: stress, neurochemistry and behavior. *Neurosci Biobehav Rev* 1989; **13:** 199–206.
5 De Nicola AF, Magariños AM, Foglia VG. Neuroendocrine regulation in experimental diabetes (Houssay Lecture). In: Rifkin H, Colwell JA, Taylor SI, eds. *Diabetes*, Amsterdam: Elsevier Science Publishers, 1991: 3–8.
6 Saravia F, Revsin Y, Gonzalez Deniselle MC, Gonzalez S, Roig P, Lima A, Homo-Delarche F, De Nicola AF. Increased astrocyte reactivity in the hippocampus of murine models of type I diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. *Brain Res* 2002; **957:** 345–353.
7 Magariños AM, McEwen BS. Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. *PNAS USA* 2000; **97:** 10056–11061.
8 Muller HW, Matthiese HP, Schmalenbach C, Schroeder WO. Glial support of CNS neuronal survival, neurite growth and regeneration. *Restorative Neurol Neurosci* 1991; **2:** 229–232.
9 Norenberg MD. Astrocyte responses to CNS injury. *J Neuropathol Exp Neurol* 1994; **53:** 213–220.
10 Reagan LP, Magariños AM, McEwen BS. Neurological changes induced by stress in streptozotocin diabetic rats. *Ann NY Acad Sci* 1999; **893:** 126–137.
11 Bestetti G, Rossi GL. Hypothalamic lesions in rats with long-term streptozotocin-induced diabetes mellitus. *Acta Neurophatol* 1980; **52:** 119–127.
12 Mukai N, Hori S, Pomeroy M. Cerebral lesions in rats with streptozotocin-induced diabetes. *Acta Neuropathol (Berl)* 1980; **51:** 79–84.
13 Russel JW, Sullivan KA, Windebank AJ, Herrmann DN, Feldman EL. Neurones undergo apoptosis in animal and cell culture models of diabetes. *Neurobiol Dis* 1999; **6:** 347–363.
14 Li ZG, Zhang W, Grunberger G, Sima AAF. Hippocampal neuronal apoptosis in type I diabetes. *Brain Res* 2002; **946:** 221–231.
15 Saravia F, Revsin Y, Roig P, Lima A, Homo-Delarche F, De Nicola AF. Hippocampal alterations in streptozotocin (STZ)-diabetic mice. *Fifth International Congress of the International Society of Neuroimmunomodulation*. Montpellier, France: International Society of Neuroimmunomodulation, 2002, Abstract 13.
16 McEwen BS, Magariños AM, Reagan LP. Studies of hormone action in the hippocampal formation. Possible relevance to depression and diabetes. *J Psychsomatic Res* 2002; **53:** 883–890.
17 Piotrowski P, Wierzbicka K, Smiatek M. Neuronal death in the rat hippocampus in experimental diabetes and cerebral ischemia treated with antioxidants. *Folia Neuropathol* 2001; **39:** 147–154.
18 Taupin P, Gage F. Adult neurogenesis and neural stem cells of the central nervous system in mammals. *J Neurosci Res* 2002; **69:** 745–749.
19 Cameron AH, Woolley CS, McEwen BS, Gould E. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. *Neuroscience* 1993; **56:** 337–344.
20 Seaberg RM, van der Kooy D. Stem and progenitor cells: the premature desertion of rigorous definitions. *TINS* 2003; **26:** 125–131.
21 Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neurosci* 1994; **61:** 203–209.
22 Gould E, Tanapat P, Rydel T, Hastings N. Regulation of hippocampal neurogenesis in adulthood. *Biol Psychiatry* 2000; **48:** 715–720.
23 Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* 2002; **12:** 578–584.
24 Jackson-Guilford J, Leander JD, Nisenbaum LK. The effect of streptozotocin-induced diabetes on cell proliferation in the rat dentate gyrus. *Neurosci Lett* 2000; **293:** 91–94.
25 Amrani A, Chaouloff F, Mormede P, Dardenne M, Homo-Delarche F. Glucose, insulin, and open field responses to immobilization in nonobese diabetic (NOD) mice. *Physiol Behav* 1994; **56:** 241–246.
26 Flood JF, Mooradian AD, Morley JE. Characteristics of learning and memory in streptozocin-induced diabetic mice. *Diabetes* 1990; **39:** 1391–1398.
27 García Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. *Prog Neurobiol* 2001; **63:** 29–60.
28 McEwen BS, Akama K, Alves S, Brake SG, Bulloch K, Lee S, Li C, Yuen G, Milner TA. Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. *Proc Natl Acad Sci USA* 2001; **98:** 7093–7100.
29 Behl C. Estrogen as a neuroprotective hormone. *Nature Rev Neurosci* 2002; **3:** 433–442.
30 Diaz-Brinton R, Chen S, Montoya M, Hsieh D, Minaya J, Kim J, Chu HP. The women's health initiative estrogen replacement therapy is neurotrophic and neuroprotective. *Neurobiol Aging* 2000; **21:** 475–496.
31 Hojo Y, Hattori T, Enami T, Furukawa A, Suzuki K, Ishii H, Mukai H, Morrinson JH, Janssen W, Kominami S, Harada N, Kimoto T, Kawato S. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P450017α and P450 aromatase localized in neurons. *Proc Natl Acad Sci USA* 2004; **101:** 865–870.
32 Tanapat P, Hastings NB, Reeves AJ, Gould E. Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat 1999. *J Neurosci* 1999; **19:** 5792–5801.
33 Liu Z, Gastard M, Verina T, Bora S, Mouton PR, Koliatsos VE. Estrogens modulate experimentally induced apoptosis of granule cells in the adult hippocampus. *J Comp Neurol* 2001; **441:** 1–8.
34 Ormerod BK, Lee TT-L, Galea LAM. Estradiol initially enhances but subsequently suppresses (via adrenal steroids) granule cell proliferation in the dentate gyrus of adult female rats. *J Neurobiol* 2003; **55:** 247–260.
35 Brannvall K, Korthonen L, Lindholm D. Estrogen-receptor dependent regulation of neural stem cell proliferation and differentiation. *Mol Cell Neurosci* 2002; **21:** 512–520.
36 Shughrue PJ, Merchenthaler I. Evidence for novel estrogen binding sites in the rat hippocampus. *Neuroscience* 2000; **99:** 605–612.
37 Perez-Martin M, Azcoitia I, Trejo JL, Sierra A, Garcia-Segura LM. An antagonist of estrogen receptors blocks the induction of adult neurogenesis by insulin-like growth factor-I in the dentate gyrus of adult female rat. *Eur J Neurosci* 2003; **18:** 923–930.
38 Lux-Lantos V, Hockl O, Tesone M, Libertun C. Anterior pituitary estradiol receptors associated with the reinstatement of ovulatory cycles after lactation interruption in the rat. *Neuroendocrinology* 1994; **59:** 265–270.
39 McMahon SS, McDermott KW. Proliferation and migration of glial precursor cells in the developing rat spinal cord. *J Neurocytol* 2001; **30:** 821–828.
40 Gould E, Woolley CS, McEwen BS. Naturally occurring cell death in the developing dentate gyrus of the rat. *J Comp Neurol* 1991; **304:** 408–418.
41 Lehmann A. *Atlas Stereotaxique Du Cerveau de la Souris*. Paris: Editions Du Centre National de la Recherche Scientifique, 1974.
42 Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age related decrease of neuronal progenitor proliferation. *J Neurosci* 1996; **16:** 2027–2033.
43 Piroli GG, Lima AE, Díaz-Torga G, De Nicola AF. Biochemical parameters in the anterior pituitary during the course of

tumorigenesis induced by diethylstilbestrol treatment. *J Steroid Biochem Mol Biol* 1994; **51:** 183–189.
44 Chinoy MR, Sharma JD, Chinoy NJ. Altered structural and functional integrity of the reproductive tissues in estradiol benzoate-treated intact male albino rats. *Int J Fertil* 1984; **29:** 98–103.
45 Adeghate E, Ponery AS. The effect of 17 beta-estradiol on weight, blood glucose and plasma insulin levels in diabetic rats. *Gynecol Endocrinol* 2001; **15:** 433–438.
46 Saravia F, Gonzalez S, Roig P, Alves V, Homo-Delarche F, De Nicola AF. Diabetes increases the expression of hypothalamic neuropeptides in a spontaneous model of type I diabetes, the non-obese diabetic (NOD) mouse. *Cell Mol Neurobiol* 2001; **21:** 15–27.
47 Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage F. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 2003; **130:** 391–399.
48 Lannert H, Wirtz P, Schuhmann V, Galmbacher R. Effects of estradiol (17β) on learning, memory and cerebral energy metabolism in male rats after intracerebroventricular administration of streptozotocin. *J Neural Transm* 1998; **105:** 1045–1063.
49 Gundlah C, Kohama SG, Mirkes SJ, Garyfallou VT, Urbanski HF, Bethea CL. Distribution of estrogen receptor beta (Erbeta) mRNA in hypothalamus, midbrain and temporal lobe of spayed macaques: continued expression with hormone replacement. *Brain Res Mol Brain Res* 2000; **76:** 191–204.
50 Okano HJ, Pfaff DW, Gibbs RB. RB and Cdc2 expression in brain: correlations with 3H-thymidine incorporation and neurogenesis. *J Neurosci* 1993; **13:** 2930–2938.
51 Carlstrom L, Ke Z, Unnerstall JR, Cohen RS, Pandey SC. Estrogen modulation of the cyclic AMP response element-binding protein pathway. *Neuroendocrinology* 2001; **74:** 227–243.
52 Cardona-Gomez GP, Mendez P, DonCarlos LL, Azcoitia I, García Segura LM. Interactions of oestrogens and insulin-like growth factor-I in the brain: implications for neuroprotection. *Brain Res Rev* 2001; **37:** 320–334.
53 Anderson MF, Aberg MAI, Nilsson M, Eriksson PS. Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. *Dev Brain Res* 2002; **134:** 115–122.
54 Toung TK, Hurn PD, Traysman RJ, Sieber FE. Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type I diabetes mellitus. *Stroke* 2000; **31:** 2701–2706.
55 Garris DR. Estrogenic stimulation of hypothalamic-limbic system metabolism in ageing diabetic C57Bl/KsJ mice. *Neuroendocrinology* 1999; **69:** 424–429.