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ORIGINAL PAPER

Sitagliptin protects proliferation of neural progenitor cells in diabetic mice

Tomás P. Bachor · Melisa D. Marquioni-Ramella · Angela M. Suburo

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Abstract Sitagliptin (SIT) is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the effects of incretin hormones, such as Glucose-dependent Insulinotropic Peptide (also known as Gastric Inhibitory Polypeptide, GIP) and Glucagon-Like Peptide 1 (GLP-1). We have now evaluated the effect of SIT on proliferation of neural progenitors in diabetic mice. A condition resembling the non-obese type 2 diabetes mellitus (D2) was achieved by a combination of streptozotocin and nicotinamide (NA-STZ), whereas a type 1-like disease (D1) was provoked by STZ without NA. Non-diabetic mice received vehicle injections. Cell proliferation was estimated by bromodeoxyuridine (BrdU) incorporation in two different regions of the subventricular zone (SVZ), the largest reserve of neural stem cells in the adult brain. SIT treatment did not modify the high fasting blood glucose (BG) levels and intraperitoneal glucose tolerance test (IPGTT) of D1 mice. By contrast, in D2 mice, SIT treatment significantly reduced BG and IPGTT. Both D1 and D2 mice showed a substantial reduction of BrdU labeling in the SVZ. Remarkably, SIT treatment improved BrdU labeling in both conditions. Our findings suggest that SIT would protect proliferation of neural progenitor cells even in the presence of non-controlled diabetic alterations.

Keywords Diabetes · Neural stem cells · Neurogenesis · Gliptins · Glucagon-Like Peptide 1 · Subventricular zone

Introduction

Interventions to prevent and treat the classic microvascular and macrovascular diabetic complications have significantly increased life expectancy of diabetic patients. However, diabetes is significantly associated to dementia and cognitive impairment, both in human subjects with type 1 and type 2 diabetes mellitus (T1DM and T2DM) (Desrocher and Rovet 2004; Greenwood and Winocur 2005; Messier 2005). A recent meta-analysis estimates that the relative risk for subjects with diabetes compared with the non-diabetic population was 1.46 for Alzheimer's disease, 2.4 for vascular dementia, 1.51 for any dementia, and 1.21 for mild cognitive impairment (Cheng et al. 2012). Thus, the brain can be considered a direct target organ for diabetes (Strachan 2011): chronic hyperglycemia will promote development of cerebral microvascular disease, whereas recurrent hypoglycemia might also be deleterious to nerve cells. Diabetes not only affects glucose metabolism, insulin signaling, and mitochondrial function in the brain, but also disrupts metabolism of Aβ and tau inducing Alzheimer's disease-like pathological changes (Sato and Morishita 2014).

Furthermore, depletion of neurogenic cell niches in the hippocampal dentate gyrus and the subventricular zone (SVZ) has been shown in various experimental models of diabetes (Bachor and Suburo 2012; Beauquis et al. 2006; Jackson-Guilford et al. 2000; Lang et al. 2009). Experimental diabetes also reduces the number of surviving neurons. It has been estimated that neuronal production at 7 weeks after streptozotocin (STZ)-diabetes induction is less than 20 % of that found in healthy mice (Zhang et al. 2008). In the non-obese diabetic (NOD) mice, a spontaneous T1DM model, glial activation and a decrease in the survival of newly formed neurons can be detected even before the appearance of diabetic clinical symptoms (Beauquis et al. 2008; Saravia et al. 2002). It has also been demonstrated that hypoglycemia induces transient neurogenesis in the hippocampus, which is followed by reduction of neuroblasts (Suh et al. 2005).

The use of sitagliptin phosphate (SIT) has been approved by the US Food and Drug Administration (FDA) to improve

T. P. Bachor · M. D. Marquioni-Ramella · A. M. Suburo (✉)
Medicina Celular y Molecular, Facultad de Ciencias Biomédicas, Universidad Austral, Pilar B1629AHJ, Argentina
e-mail: amsuburo@austral.edu.ar

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glycemic control in T2DM patients, alone or in combination with metformin or thiazolidinediones (Zerilli and Pyon 2007). As other members of the gliptin family, SIT is a dipeptidyl peptidase-4 (DPP-4) inhibitor that enhances the effects of incretin hormones. These hormones are responsible for the difference between the insulin response evoked by an oral glucose load and that elicited by an intravenous glucose injection producing the same plasma glucose concentrations (Creutzfeldt and Ebert 1985). Response to the main incretin hormones, Glucagon-Like Peptide 1 (GLP-1) and Glucose-dependent Insulinotropic Peptide (also known as Gastric Inhibitory Polypeptide, GIP) is deficient in T2DM patients (Nauck et al. 2004). Hyperglycemia attenuates islet GIP receptor expression, but GLP-1 can still stimulate insulin secretion in T2DM. Thus, GLP-1 has become the parent compound for incretin-directed therapeutics in T2DM (Drucker and Nauck 2006). Recent investigations have shown that SIT also has a beneficial effect on T1DM patients (Ellis et al. 2011; Zhao et al. 2014), suggesting that incretins may also be involved in this condition. Moreover, SIT and other gliptins afford neuroprotection in T1DM and T2DM mice models (Darsalia et al. 2013, 2014; Kosaraju et al. 2013; Pintana et al. 2013; Sakr 2013).

We studied the effect of SIT on neuroprogenitors in the mouse SVZ, as this neurogenic niche contains the largest reserve of proliferating neural stem cells (Codega et al. 2014; Gil-Perotin et al. 2013). A condition resembling the non-obese T2DM was achieved by a combination of STZ and nicotinamide (NA), whereas a T1DM-like disease was provoked by STZ without NA (Nakamura et al. 2006). In the text, we will refer to these experimental conditions as D2 and D1.

Methods

Animals

Male C57Bl/6J mice (5 to 7-weeks old) were bred and cared according to the "U.S. Public Health Service's Policy on Humane Care Research Use of Laboratory Animals (PHS policy)", with food and water ad libitum. Procedures were approved by the Animal Care Committee of our Institution. To reduce stress and other possible environmental confounders, experiments were carried out in males from litters of the same age that were weaned to a single large cage. Mice in group 1 were 7 weeks-old ($n = 12$), whereas those in group 2 had 5 weeks ($n = 12$) at the beginning of the experiment.

Blood glucose measurements

Blood glucose (BG) was measured in a small blood drop from the tail vein using a Lifescan© Surestep® (Johnson & Johnson Medical, Argentina) glucose meter and reactive strips. Intraperitoneal Glucose Tolerance Tests (IPGTTs) were done 2 days before the experimental end-point. Fasting BG was determined 6 h after food withdrawal (Andrikopoulos et al. 2008), without water restriction. After ip glucose injection (2 g/kg), BG was evaluated at 15, 30 and 60 min. Glucose tolerance was calculated as the area under the curve (AUC) for the first 60 min.

Diabetes induction

In group D1, diabetes was induced by STZ (100 mg/kg, ip; Sigma-Aldrich Co., St. Louis) diluted in citrate buffer (pH 4.5). Mice were fasted during 4 h before injection. The procedure was repeated after 2 days (day 3). Mice in group D2 received nicotinamide (NA; 120 mg/kg, ip; Sigma-Aldrich Co., St. Louis) 15 min before STZ. Both injections were repeated after 2 days. Non-diabetic (Non-D) animals received the same volumes of citrate buffer.

Beginning on day 4, and until day 18, half of the diabetic animals, groups D1 + S and D2 + S, received daily SIT injections (Januvia, Merck, Sharp & Dohme, Argentina; 100 mg/kg/day, ip) (Jackson and Mi 2008). Non-D and untreated diabetic mice (D1 and D2) were given the same volumes of saline solution. As an additional control, a different group of healthy Non-D mice received saline ($n = 4$) or SIT ($n = 4$) injections during 15 days.

Bromodeoxyuridine incorporation and immunohistochemistry

Twenty-four hours before fixation, mice received a single 5-bromo-2′-deoxyuridine (BrdU; 150 mg/kg, ip; Sigma-Aldrich Co., St. Louis) injection. On day 19, mice were deeply anesthetized and perfused as previously described (Castañeda et al. 2011). A mouse stainless steel coronal brain matrix (David Kopf Instruments, Tujunga, CA) was used to slice the brain. Slices between +1.70 and −0.80 AP (Paxinos and Franklin 2001) were embedded in OCT and snap frozen in N_2-cooled isopentane. Each frozen block contained slices from two brains. Seriate cryosections (10 μm) were mounted on glass slides that were stained with Cresyl Violet or processed for BrdU immunohistochemistry. DNA denaturalization was carried out in 2N HCl plus 5 % Triton-X100 (Sigma-Aldrich Co., St. Louis MO) during 60 min at room temperature. Sections were first washed in 0.1 M sodium borate buffer (pH 8.5) and then phosphate-buffered saline (PBS). They were then incubated with a rat monoclonal anti-BrdU (Abcam® ab6326). Detection was made with biotinylated secondary antibodies, the avidin-biotin peroxidase complex (Vectastain® Elite ABC peroxidase kit, Vector Labs, Burlingame, CA) and nickel-enhanced diaminobencidine reaction (Castañeda et al. 2011).

Image analysis and statistics

For quantification of BrdU+ nuclei, we immunostained each 10th slide and selected those containing sections between +1.00 and bregma (Paxinos and Franklin 2001). In the selected sections (4–6 per brain), the regions of interest (ROIs) around the lateral ventricle were photographed at 40×. A blind operator examined the photomontages and measured immunoreactive areas in the lateral wall (LW) and the subcallosal enlargement (SCE). The LW region included all labeled nuclei adjacent to the ventricular wall along a 400 μm segment ventral to the dorsolateral angle of the lateral ventricle. The SCE was defined as the region underlying the corpus callosum (CC) that tapers into the Rostral Migratory Stream (RMS). The SCE included all labeled nuclei placed between the dorsolateral angle and an arbitrary line placed at 400 μm from that angle (Fig. 1). An arbitrary threshold value was defined in order to convert all pixels lighter than this value into white and pixels darker that this value into black. The resulting segregated area (dark pixels only) was measured by an automatic action (Adobe Photoshop© CS6). In all cases the same threshold value was used. The results are given as BrdU pixels/ROI.

GraphPad Prism 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) was used for statistical comparisons and calculations of area under the curve (AUC). Data was compared by ANOVA followed by Newman-Keuls multiple comparison tests.

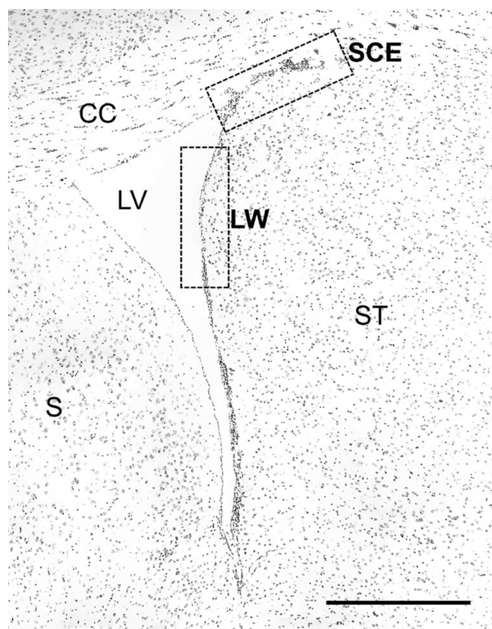

Fig. 1 A Cresyl Violet-stained brain coronal section showing the lateral ventricle and the regions of interest used for BrdU immunoreactivity scoring. *Dashed rectangles* show the regions areas used for quantification. We scored all immunoreactive nuclei placed along a 400 μm distance beginning at the dorsalmost border of the LW region, or at the medialmost border of the SCE. Abbreviations for this and other figures: *CC* corpus callosum, *LV* lateral ventricle, *LW* lateral wall, *S* septum, *ST* striatum, *SAL* saline, *SCE* subcallosal enlargment, *SIT* sitagliptin. Calibration bar, 400 μm

Results

Metabolic outcomes

Diabetic mice weighted less than the corresponding Non-D animals, in both D1 and D2 groups (Table 1). Initially, all mice showed BG levels in the normal range for this strain. At the end of the experiment, D1 mice, with or without SIT, showed very high fasting BG levels, exceeding the measuring limit of glucose strips.

Fasting BG levels were only moderately increased in the D2 group. After SIT treatment (D2 + SIT), fasting BG levels showed no statistical significant difference with BG values in Non-D mice (Table 1). An abnormally high IPGTT appeared in D2 mice, whereas Non-D and SIT-treated D2 mice showed similar values (Table 2).

BrdU incorporation in the subventricular zone

As has been extensively described (Landgren and Curtis 2011), BrdU labeled nuclei were selectively located along the lateral wall (LW) of the lateral ventricle. A significant number, however, also appeared along the dorsal wall (Castañeda et al. 2010; Ventura and Goldman 2007). A cluster of labeled nuclei showed at the SCE and extended to the RMS. SIT treatment for 18 days did not modify BrdU labeling patterns in the SVZ of healthy Non-D mice (Fig. 2).

Few labeled nuclei appeared in the ventricular LW of D1 and D2 mice. After SIT treatment, however, the amount of labeled nuclei was much higher than in the corresponding diabetic animals (Fig. 3). The SCE showed a similar pattern, with a large decrease of labeled nuclei in diabetic (D1 and D2)

Table 1 Body weights and fasting glucose levels at 18 days post-diabetes induction

	Non-D	D1	D1 + S
Body weight	32.0 ± 0.4	28.4 ± 0.2***	26.5 ± 0.5***
Fasting blood glucose	159.3 ± 12.3	>486.0	>486.0
	Non-D	D2	D2 + S
Body weight	26.8 ± 0.8	21.5 ± 0.9***	20.1 ± 0.5***
Fasting blood glucose	126.6 ± 9.8	257.5 ± 32.7**	171.8 ± 26.2$^{\#}$

One Way ANOVA followed by Newman-Keuls Multiple Comparison Test was used for statistical comparisons. Differences in weight for all diabetic mice were statistically significant ($p < 0.001$). Fasting BG levels in D1 mice exceeded the measurement range. In D2 mice they were significantly higher than in control mice ($p < 0.01$). In SIT-treated D2 mice (D2 + S) fasting BG levels were lower than in untreated D2 mice ($p < 0.05$), but were not different from fasting BG levels in controls

Table 2 Intraperitoneal glucose tolerance test

AUC_{60min}	D1	D2
Non-diabetic	14,060 ± 180	10,370 ± 261***
Diabetic	>29,000	18,690 ± 1959
Diabetic + SIT	>29,000	12,380 ± 604**

Results are given as the AUC for the first 60 min of the IPGTT curve after glucose overload. AUCs for treated and untreated D1 mice are estimative. AUCs for D2 mice were significantly higher than AUCs in Non-D ($p < 0.001$) and SIT-treated D2 mice ($p < 0.01$). No statistically significant differences appeared between Non-D and SIT-treated D2 mice

animals and an almost normal labeling pattern in SIT-treated diabetic mice (Fig. 4). Observations were confirmed by quantitative analysis. BrdU labeling in D1 and D2 mice showed very low values, statistically different from those of Non-D mice. Decrease was almost nil in the LW and the SCE of SIT-treated diabetic mice. In D1 + SIT mice, BrdU immunoreactivity was still significantly lower than in Non-D animals. By contrast, BrdU labeling in D2 + SIT mice was not different from that in Non-D mice (Fig. 5).

Discussion

Streptozotocin-induced diabetes and sitagliptin

Under the conditions of our experiment, mice treated with two STZ doses (D1 mice) developed a severe non-obese T1DM-like condition, with very high fasting BG levels and abnormal IPGTTs. Mice treated with NA followed by STZ (D2 mice) showed a moderate increase in fasting BG with an 80 % amplification of the IPGTT curve area. Thus, metabolic

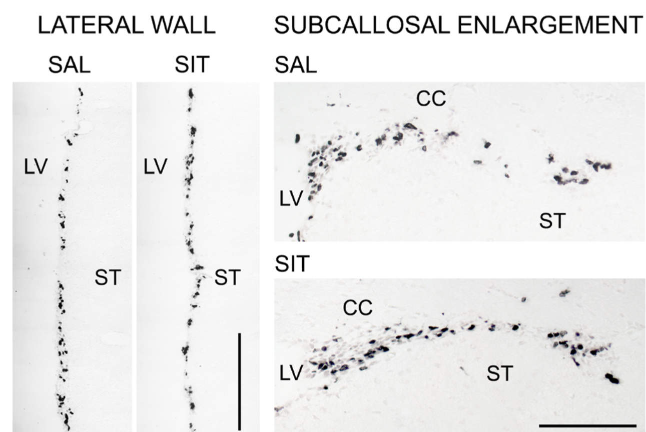

Fig. 2 BrdU immunostaining in the SVZ of healthy Non-D mice receiving SAL or SIT during 15 days. *Left*, no differences between mice receiving SAL- or SIT could be detected along the ventricular lateral wall. *Right*, the SCE of SAL and SIT-treated mice showed similar BrdU immunostaining patterns. Calibration bars, 100 μm

conditions of D2 mice resembled those of a non-obese T2DM-like condition (American Diabetes 2010). Their BG levels and glucose tolerance were within the range of those previously reported for NA-STZ-induced diabetes in C57Bl6 mice (Shimizu et al. 2012). As shown in the ICR strain, NA-STZ reduces the amount of pancreatic insulin to about 50 % of normal levels, with little insulin resistance (Tahara et al. 2008).

SIT treatment did not significantly modify metabolic parameters in D1 mice. By contrast, fasting BG levels and the IPGTT curve areas were significantly reduced in SIT-treated D2 mice. As has been shown by others, SIT can preserve the β-cell mass in several T2DM-like models (Mu et al. 2009; Poucher et al. 2012). In our experiments, reversal of metabolic diabetic signs was only observed in D2 mice. Remarkably, it could be observed at less than 3 weeks of daily SIT administration. Functional recovery, however, has also been observed after a single SIT administration, given one week after NA-STZ diabetes induction (Tahara et al. 2008). Islet protection under T1DM conditions might require treatment before diabetes induction, since des-fluoro-sitagliptin oral administration beginning 14 days before STZ administration (5 × 50 mg/kg) reduces islet cell death and hyperglycemia (Takeda et al. 2012).

Diabetes and proliferation of adult neuroprogenitors

Similar decreases of BrdU incorporation were evident in the LW of the lateral ventricle and in the SCE, both in D1 and D2 mice. Neurogenesis impairment in STZ-diabetic mice has been previously shown (Beauquis et al. 2006; Jackson-Guilford et al. 2000; Saravia et al. 2004). However, the effects of T2DM on neurogenic niches are far from clear. In the Zucker diabetic fatty rat, a T2DM-like model, cell proliferation and neuronal differentiation are always lower than in Non-D rats, even after blood glucose levels have been normalized by exercise (Hwang et al. 2010; Yi et al. 2009). By contrast, in the Goto-Kakizaki rat, neuroprogenitor proliferation increases together with a decrease in survival of newly-born neurons (Beauquis et al. 2010; Lang et al. 2009). A proliferation increase has also been reported in the SVZ of db/db mice (Ramos-Rodriguez et al. 2014). Our observations suggest that the proliferation decrease would not solely depend on glucose levels, since mice with dissimilar fasting BG levels and marked differences in glucose tolerance showed similar BrdU incorporation patterns.

Under the conditions of our experiments, the single injection of BrdU would label a cohort of cells that were in S phase at the time of the injection (Nowakowski et al. 1989) and progressed through the cell cycle during the following 24 h. Activated neural stem cells (B1 cells) generate intermediate progenitor cells (C cells). These cells divide symmetrically approximately three times before becoming migratory neuroblasts (A cells), which divide one or two more times

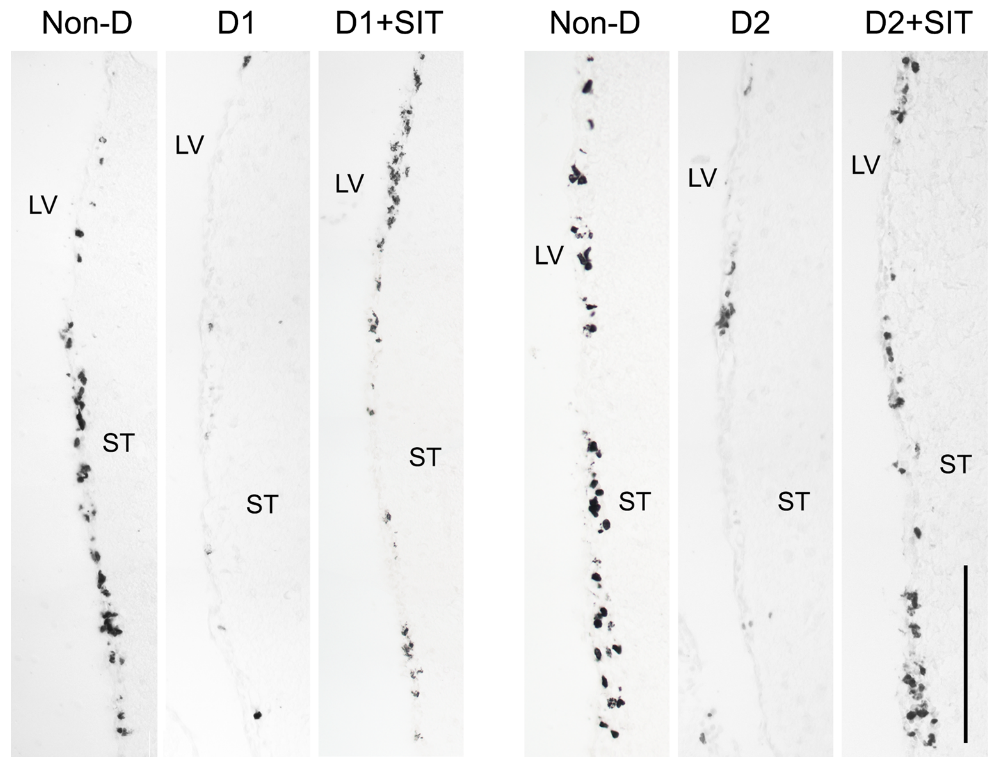

Fig. 3 BrdU immunoreactivity in the ventricular lateral wall of the different experimental groups. Immunoreactive nuclei were randomly distributed along the ventricular wall, close to the striatum (ST). A large reduction of BrdU immunoreactivity can be observed in D1 and D2 diabetic animals. After SIT treatment, BrdU immunostaining was almost identical to that observed in the Non-D animals. Calibration bar, 100 μm

while migrating to the olfactory bulb (Ponti et al. 2013). Therefore, the observed decrease in BrdU nuclear incorporation most likely reflects a depletion of these cycling subpopulations. Their decline could in turn obey to cell death (Mansouri et al. 2012) and/or lengthening of the cell cycle. Proliferation impairment in the diabetic neurogenic niches has been associated with elevated glucocorticoid levels and to down-regulation of brain-derived neurotrophic factor (BDNF) expression (Guo et al. 2010). BDNF also restores proliferation of neural progenitors affected by products of lipid peroxidation (Park et al. 2010). Exercise and dietary energy restriction, which have antidiabetic effects, can enhance neurogenesis in rodents (Lee et al. 2002; Stranahan et al. 2006; van Praag et al. 1999).

Sitagliptin, the diabetic milieu and neuroprogenitor cell proliferation

After SIT treatment, D1 and D2 showed little or no impairment of BrdU incorporation. Fasting BG levels and glucose

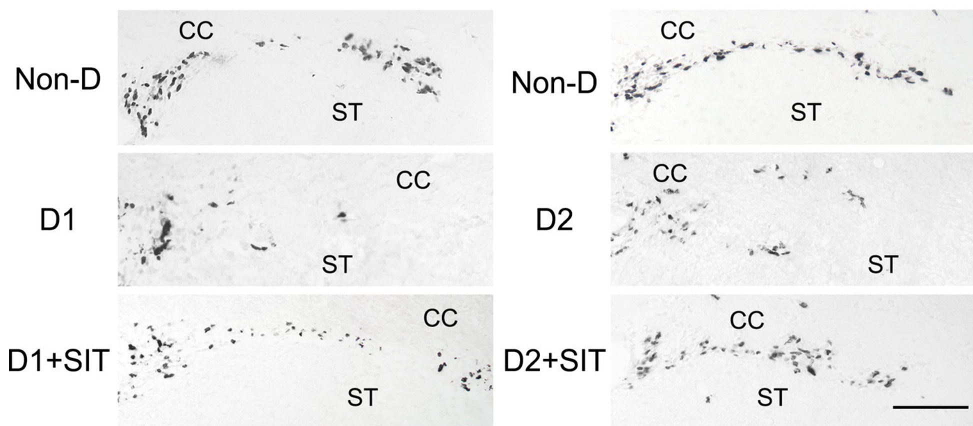

Fig. 4 The subcallosal enlargement, placed between the corpus callosum (CC) and the striatum (ST), showed numerous BrdU immunoreactive nuclei. These nuclei were clustered close to the dorsolateral angle of the lateral ventricle (left border of all images), and their number tapered towards the periphery. BrdU immunoreactivity almost disappeared in D1 and D2 diabetic animals. By contrast, in SIT-treated diabetic animals, BrdU immunoreactivy appeared close to the normal range. Calibration bar, 100 μm

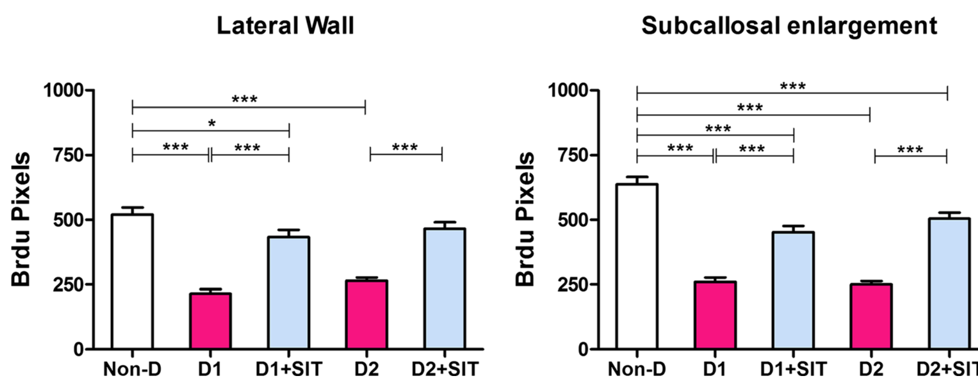

Fig. 5 Quantification of BrdU immunoreactive areas in the SVZ of D1 and D2 mice. Values from Non-D mice from D1 and D2 experiments were not statistically different and had been pooled for this ANOVA analysis. *Right*, in the lateral wall, both D1 and D2 reduced labeling to about half of the non-diabetic values. Reduction was minimal in D1 SIT-treated mice, whereas values in D2 SIT-treated mice were not different from those in Non-D mice. *Left*, in the subcallosal enlargement, decrease was about 60 % for both D1 and D2. After SIT treatment, a 30 % decrease appeared in D1 mice. A smaller decrease appeared in D2 + SIT mice but it was not statistically different from values in Non-D mice

tolerance in D2 + SIT mice were not statistically different from those of control mice, suggesting that maintenance of neuroprogenitor proliferation in these animals might be ascribed to amelioration of the diabetic condition. However, SIT also protected neuroprogenitor proliferation in D1 mice, without a parallel modification of fasting BG levels and glucose tolerance. Thus, maintenance of normal proliferation in the SVZ neurogenic niche might represent a direct effect of DPP-4 inhibition in the brain.

A SIT-induced increase in brain GLP-1 levels (D'Amico et al. 2010) would most likely be involved, since several beneficial effects of GLP-1 analogs on the brain have been described. These analogs prevent brain damage in a diabetic background (Chen et al. 2011; Darsalia et al. 2012; Hamilton et al. 2011; Hunter and Holscher 2012), and provide neuroprotection in models of Alzheimer's and Huntington's diseases (Li et al. 2010; Martin et al. 2009; Perry and Greig 2005).

On the other hand, gliptin effects might be mediated by other peptides, with or without incretin properties (Omar and Ahren 2014). GIP, one of the incretins, has been related to proliferation of neural progenitor cells in the hippocampus (Faivre et al. 2012; Nyberg et al. 2005). Among non-incretins, the stromal-derived factor-1α (CXCL12) is another DPP-4 substrate that has been associated with migration of neural stem cells (Kokovay et al. 2010). The pituitary adenylate cyclase-activating polypeptide (PACAP) and the vasoactive intestinal peptide (VIP), also targeted by DPP-4 (Jungraithmayr et al. 2010; Zhu et al. 2003), could also regulate neural stem cell proliferation and differentiation (Hirose et al. 2006; Mercer et al. 2004; Scharf et al. 2008). In addition, chronic treatment with alogliptin increases BDNF in the cortex and the thalamostriatum (Yang et al. 2013).

Various gliptins have been shown to improve cognitive function in T2DM rodents (Kosaraju et al. 2013; Pintana et al. 2013; Sakr 2013). In addition, alogliptin and linagliptin prophylactic treatment tend to reduce experimental brain infarct volume in diabetic and Non-D animals (Darsalia et al. 2013; Yang et al. 2013). Prophylactic alogliptin treatment before experimental stroke significantly increases BDNF levels in the brain cortex (Yang et al. 2013). Similarly, prophylactic linagliptin enhances the number of striatal surviving neurons after experimental stroke in T2DM mice (Darsalia et al. 2013). This treatment also increases KI67 in the SVZ in the ischemic hemisphere but not in the contralateral neurogenic niche (Darsalia et al. 2014). This finding, together with our present observations in Non-D mice, indicates that gliptins would provide proliferation support to a damaged neurogenic niche but would produce no effects in a healthy brain. Further studies are required to understand the mechanistic pathways involved in these phenomena.

Normal BrdU incorporation in SIT-treatead diabetic mice without concomitant effects on BG levels and glucose tolerance suggests that neuroprogenitors can still proliferate under hyperglycemia. This phenomenon has been previously shown in diabetic rats pre-treated with estradiol and fluoxethine (Beauquis et al. 2006; Saravia et al. 2004) and, more recently, with indomethacin (Ho et al. 2014).

Protection of neural stem proliferation without prior correction of the diabetic milieu might be of medical interest. It might help to preserve mental functions in chronic- and long-standing diabetes, and perhaps also during acute diabetes bouts occurring in relation to sepsis and vascular complications.

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References

American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33(Suppl 1):S62–S69. doi:10.2337/dc10-S062

Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J (2008) Evaluating the glucose tolerance test in mice. Am J Physiol Endocrinol Metab 295:E1323–E1332. doi:10.1152/ajpendo.90617.2008

Bachor TP, Suburo AM (2012) Neural stem cells in the diabetic brain. Stem Cells Int 2012:820790. doi:10.1155/2012/820790

Beauquis J, Roig P, Homo-Delarche F, De Nicola A, Saravia F (2006) Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. Eur J Neurosci 23:1539–1546. doi:10.1111/j.1460-9568.2006.04691.x

Beauquis J, Saravia F, Coulaud J, Roig P, Dardenne M, Homo-Delarche F, De Nicola A (2008) Prominently decreased hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mouse. Exp Neurol 210:359–367. doi:10.1016/j.expneurol.2007.11.009

Beauquis J et al. (2010) Hippocampal neurovascular and hypothalamic-pituitary-adrenal axis alterations in spontaneously type 2 diabetic GK rats. Exp Neurol 222:125–134. doi:10.1016/j.expneurol.2009.12.022

Castañeda MM, Cubilla MA, López-Vicchi MM, Suburo AM (2010) Endothelinergic cells in the subependymal region of mice. Brain Res 1321:20–30. doi:10.1179/016164111X12881719352219

Castañeda MM, Cubilla MA, Bachor T, Suburo AM (2011) Endothelinergic signaling during recovery of brain cortical lesions. Neurol Res 33:137–144. doi:10.1179/016164111X12881719352219

Chen S, Liu AR, An FM, Yao WB, Gao XD (2011) Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendin-4. Age (Dordr) 34:1121–1124. doi:10.1007/s11357-011-9303-8

Cheng G, Huang C, Deng H, Wang H (2012) Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Intern Med J 42:484–491. doi:10.1111/j.1445-5994.2012.02758.x

Codega P, Silva-Vargas V, Paul A, Maldonado-Soto AR, Deleo AM, Pastrana E, Doetsch F (2014) Prospective identification and purification of quiescent adult neural stem cells from their in vivo niche. Neuron 82:545–559. doi:10.1016/j.neuron.2014.02.039

Creutzfeldt W, Ebert R (1985) New developments in the incretin concept. Diabetologia 28:565–573

D'Amico M, Di Filippo C, Marfella R, Abbatecola AM, Ferraraccio F, Rossi F, Paolisso G (2010) Long-term inhibition of dipeptidyl peptidase-4 in Alzheimer's prone mice. Exp Gerontol 45:202–207. doi:10.1016/j.exger.2009.12.004

Darsalia V et al. (2012) Glucagon-like peptide-1 receptor activation reduces ischaemic brain damage following stroke in Type 2 diabetic rats. Clin Sci (Lond) 122:473–483. doi:10.1042/CS20110374

Darsalia V et al. (2013) The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. Diabetes 62:1289–1296. doi:10.2337/db12-0988

Darsalia V et al. (2014) Linagliptin enhances neural stem cell proliferation after stroke in type 2 diabetic mice. Regul Pept 190-191:25–31. doi:10.1016/j.regpep.2014.05.001

Desrocher M, Rovet J (2004) Neurocognitive correlates of type 1 diabetes mellitus in childhood. Child Neuropsychol 10:36–52. doi:10.1076/chin.10.1.36.26241

Drucker DJ, Nauck MA (2006) The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 368:1696–1705. doi:10.1016/S0140-6736(06)69705-5

Ellis SL, Moser EG, Snell-Bergeon JK, Rodionova AS, Hazenfield RM, Garg SK (2011) Effect of sitagliptin on glucose control in adult patients with Type 1 diabetes: a pilot, double-blind, randomized, crossover trial. Diabet Med 28:1176–1181. doi:10.1111/j.1464-5491.2011.03331.x

Faivre E, Hamilton A, Holscher C (2012) Effects of acute and chronic administration of GIP analogues on cognition, synaptic plasticity and neurogenesis in mice. Eur J Pharmacol 674:294–306. doi:10.1016/j.ejphar.2011.11.007

Gil-Perotin S, Duran-Moreno M, Cebrian-Silla A, Ramirez M, Garcia-Belda P, Garcia-Verdugo JM (2013) Adult neural stem cells from the subventricular zone: a review of the neurosphere assay. Anat Rec 296:1435–1452. doi:10.1002/ar.22746

Greenwood CE, Winocur G (2005) High-fat diets, insulin resistance and declining cognitive function. Neurobiol Aging 26:S42–S45. doi:10.1016/j.neurobiolaging.2005.08.017

Guo J et al. (2010) Impaired neural stem/progenitor cell proliferation in streptozotocin-induced and spontaneous diabetic mice. Neurosci Res 68:329–336. doi:10.1016/j.neures.2010.08.012

Hamilton A, Patterson S, Porter D, Gault VA, Holscher C (2011) Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. J Neurosci Res 89:481–489. doi:10.1002/jnr.22565

Hirose M et al. (2006) Inhibition of self-renewal and induction of neural differentiation by PACAP in neural progenitor cells. Ann N Y Acad Sci 1070:342–347. doi:10.1196/annals.1317.042

Ho N, Brookshire BR, Clark JE, Lucki I (2014) Indomethacin reverses decreased hippocampal cell proliferation in streptozotocin-induced diabetic mice. Metab Brain Dis. doi:10.1007/s11011-014-9611-7

Hunter K, Holscher C (2012) Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. BMC Neurosci 13:33. doi:10.1186/1471-2202-13-33

Hwang IK, Yi SS, Song W, Won MH, Yoon YS, Seong JK (2010) Effects of age and treadmill exercise in chronic diabetic stages on neuroblast differentiation in a rat model of type 2 diabetes. Brain Res 1341:63–71. doi:10.1016/j.brainres.2009.12.009

Jackson-Guilford J, Leander JD, Nisenbaum LK (2000) The effect of streptozotocin-induced diabetes on cell proliferation in the rat dentate gyrus. Neurosci Lett 293:91–94

Jackson EK, Mi Z (2008) Sitagliptin augments sympathetic enhancement of the renovascular effects of angiotensin II in genetic hypertension. Hypertension 51:1637–1642. doi:10.1161/HYPERTENSIONAHA.108.112532

Jungraithmayr W et al. (2010) Inhibition of CD26/DPP IV attenuates ischemia/reperfusion injury in orthotopic mouse lung transplants: the pivotal role of vasoactive intestinal peptide. Peptides 31:585–591. doi:10.1016/j.peptides.2009.12.012

Kokovay E et al. (2010) Adult SVZ lineage cells home to and leave the vascular niche via differential responses to SDF1/CXCR4 signaling. Cell Stem Cell 7:163–173. doi:10.1016/j.stem.2010.05.019

Kosaraju J, Murthy V, Khatwal RB, Dubala A, Chinni S, Muthureddy Nataraj SK, Basavan D (2013) Vildagliptin: an anti-diabetes agent ameliorates cognitive deficits and pathology observed in streptozotocin-induced Alzheimer's disease. J Pharm Pharmacol 65:1773–1784. doi:10.1111/jphp.12148

Landgren H, Curtis MA (2011) Locating and labeling neural stem cells in the brain. J Cell Physiol 226:1–7. doi:10.1002/jcp.22319

Lang BT, Yan Y, Dempsey RJ, Vemuganti R (2009) Impaired neurogenesis in adult type-2 diabetic rats. Brain Res 1258:25–33. doi:10.1016/j.brainres.2008.12.026

Lee J, Duan W, Mattson MP (2002) Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in

part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J Neurochem 82:1367–1375

Li Y et al. (2010) GLP-1 receptor stimulation reduces amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease. J Alzheimers Dis 19:1205–1219. doi:10.3233/JAD-2010-1314

Mansouri S, Ortsater H, Pintor Gallego O, Darsalia V, Sjoholm A, Patrone C (2012) Pituitary adenylate cyclase-activating polypeptide counteracts the impaired adult neural stem cell viability induced by palmitate. J Neurosci Res 90:759–768. doi:10.1002/jnr.22803

Martin B et al. (2009) Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. Diabetes 58:318–328. doi:10.2337/db08-0799

Mercer A et al. (2004) PACAP promotes neural stem cell proliferation in adult mouse brain. J Neurosci Res 76:205–215. doi:10.1002/jnr.20038

Messier C (2005) Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. Neurobiol Aging 26(Suppl 1):26–30. doi:10.1016/j.neurobiolaging.2005.09.014

Mu J et al. (2009) Inhibition of DPP-4 with sitagliptin improves glycemic control and restores islet cell mass and function in a rodent model of type 2 diabetes. Eur J Pharmacol 623:148–154. doi:10.1016/j.ejphar.2009.09.027

Nakamura T, Terajima T, Ogata T, Ueno K, Hashimoto N, Ono K, Yano S (2006) Establishment and pathophysiological characterization of type 2 diabetic mouse model produced by streptozotocin and nicotinamide. Biol Pharm Bull 29:1167–1174

Nauck MA, Baller B, Meier JJ (2004) Gastric inhibitory polypeptide and glucagon-like peptide-1 in the pathogenesis of type 2 diabetes. Diabetes 53(Suppl 3):S190–S196

Nowakowski RS, Lewin SB, Miller MW (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol 18:311–318

Nyberg J et al. (2005) Glucose-dependent insulinotropic polypeptide is expressed in adult hippocampus and induces progenitor cell proliferation. J Neurosci 25:1816–1825. doi:10.1523/JNEUROSCI.4920-04.2005

Omar B, Ahren B (2014) Pleiotropic mechanisms for the glucose-lowering action of DPP-4 inhibitors. Diabetes 63:2196–2202. doi:10.2337/db14-0052

Park HR, Park M, Choi J, Park KY, Chung HY, Lee J (2010) A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. Neurosci Lett 482:235–239. doi:10.1016/j.neulet.2010.07.046

Paxinos G, Franklin KBJ (2001) The mouse brain in stereotaxic coordinates. Academic, San Diego

Perry T, Greig NH (2005) Enhancing central nervous system endogenous GLP-1 receptor pathways for intervention in Alzheimer's disease. Curr Alzheimer Res 2:377–385

Pintana H, Apaijai N, Chattipakorn N, Chattipakorn SC (2013) DPP-4 inhibitors improve cognition and brain mitochondrial function of insulin-resistant rats. J Endocrinol 218:1–11. doi:10.1530/JOE-12-0521

Ponti G, Obernier K, Guinto C, Jose L, Bonfanti L, Alvarez-Buylla A (2013) Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. Proc Natl Acad Sci U S A 110:E1045–E1054. doi:10.1073/pnas.1219563110

Poucher SM, Cheetham S, Francis J, Zinker B, Kirby M, Vickers SP (2012) Effects of saxagliptin and sitagliptin on glycaemic control and pancreatic beta-cell mass in a streptozotocin-induced mouse model of type 2 diabetes. Diabetes Obes Metab 14:918–926. doi:10.1111/j.1463-1326.2012.01619.x

Ramos-Rodriguez JJ et al. (2014) Central proliferation and neurogenesis is impaired in type 2 diabetes and prediabetes animal models. PLoS One 9:e89229. doi:10.1371/journal.pone.0089229

Sakr HF (2013) Effect of sitagliptin on the working memory and reference memory in type 2 diabetic Sprague–Dawley rats: possible role of adiponectin receptors 1. J Physiol Pharmacol 64:613–623

Saravia F, Revsin Y, Lux-Lantos V, Beauquis J, Homo-Delarche F, De Nicola AF (2004) Oestradiol restores cell proliferation in dentate gyrus and subventricular zone of streptozotocin-diabetic mice. J Neuroendocrinol 16:704–710. doi:10.1111/j.1365-2826.2004.01223.x

Saravia FE et al. (2002) Increased astrocyte reactivity in the hippocampus of murine models of type 1 diabetes: the nonobese diabetic (NOD) and streptozotocin-treated mice. Brain Res 957:345–353

Sato N, Morishita R (2014) Brain alterations and clinical symptoms of dementia in diabetes: abeta/tau-dependent and independent mechanisms. Front Endocrinol 5:143. doi:10.3389/fendo.2014.00143

Scharf E, May V, Braas KM, Shutz KC, Mao-Draayer Y (2008) Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) regulate murine neural progenitor cell survival, proliferation, and differentiation. J Mol Neurosci 36:79–88. doi:10.1007/s12031-008-9097-z

Shimizu R, Sakazaki F, Okuno T, Nakamuro K, Ueno H (2012) Difference in glucose intolerance between C57BL/6 J and ICR strain mice with streptozotocin/nicotinamide-induced diabetes. Biomed Res 33:63–66

Strachan MW (2011) R D Lawrence Lecture 2010. The brain as a target organ in Type 2 diabetes: exploring the links with cognitive impairment and dementia. Diabet Med 28:141–147. doi:10.1111/j.1464-5491.2010.03199.x

Stranahan AM, Khalil D, Gould E (2006) Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci 9:526–533. doi:10.1038/Nn1668

Suh SW et al. (2005) Hypoglycemia induces transient neurogenesis and subsequent progenitor cell loss in the rat hippocampus. Diabetes 54:500–509

Tahara A, Matsuyama-Yokono A, Nakano R, Someya Y, Shibasaki M (2008) Effects of antidiabetic drugs on glucose tolerance in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic mice. Horm Metab Res 40:880–886. doi:10.1055/s-0028-1087167

Takeda Y et al. (2012) Reduction of both beta cell death and alpha cell proliferation by dipeptidyl peptidase-4 inhibition in a streptozotocin-induced model of diabetes in mice. Diabetologia 55:404–412. doi:10.1007/s00125-011-2365-4

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96:13427–13431. doi:10.1073/pnas.96.23.13427

Ventura RE, Goldman JE (2007) Dorsal radial glia generate olfactory bulb interneurons in the postnatal murine brain. J Neurosci 27:4297–4302. doi:10.1523/JNEUROSCI.0399-07.2007

Yang D, Nakajo Y, Iihara K, Kataoka H, Yanamoto H (2013) Alogliptin, a dipeptidylpeptidase-4 inhibitor, for patients with diabetes mellitus type 2, induces tolerance to focal cerebral ischemia in non-diabetic, normal mice. Brain Res 1517:104–113. doi:10.1016/j.brainres.2013.04.015

Yi SS et al. (2009) Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. Neurochem Res 34:1039–1046. doi:10.1007/s11064-008-9870-y

Zerilli T, Pyon EY (2007) Sitagliptin phosphate: a DPP-4 inhibitor for the treatment of type 2 diabetes mellitus. Clin Ther 29:2614–2634. doi:10.1016/j.clinthera.2007.12.034

Zhang WJ, Tan YF, Yue JT, Vranic M, Wojtowicz JM (2008) Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. Acta Neurol Scand 117:205–210. doi:10.1111/j.1600-0404.2007.00928.x

Zhao Y et al. (2014) Dipeptidyl peptidase 4 inhibitor sitagliptin maintains beta-cell function in patients with recent-onset latent autoimmune diabetes in adults: one year prospective study. J Clin Endocrinol Metab 99:E876–E880. doi:10.1210/jc.2013-3633

Zhu L et al. (2003) The role of dipeptidyl peptidase IV in the cleavage of glucagon family peptides: in vivo metabolism of pituitary adenylate cyclase activating polypeptide-(1-38). J Biol Chem 278:22418–22423. doi:10.1074/jbc.M212355200