Neuronal Plasticity and Antidepressants in the Diabetic Brain

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The hippocampus, a limbic structure linked to higher brain functions, appears vulnerable in diabetic subjects that have a higher risk of stroke, dementia, and cognitive decline. The dentate gyrus (DG) of the hippocampus is one of the limited neurogenic brain areas during adulthood; neurons born in the DG are involved in some types of learning and memory processes. We found a decrease in the ability for proliferation and neuronal differentiation of newborn cells, measured by bromodeoxyuridine incorporation in the DG, from streptozotocin-induced diabetic mice. The hilar region, formed by mature neurons presenting higher sensitivity to brain damage, showed a reduced neuronal density in diabetic mice with respect to vehicle-treated mice. Interestingly, in a spontaneous model of type 1 diabetes, we corroborated a decrease in the rate of neurogenesis in the nonobese diabetic mice compared to control strains, and this reduction was also found during the prediabetic stage. The antidepressant fluoxetine administered over a period of 10 days to diabetic mice was effective in preventing changes in proliferation and differentiation of new neurons. Confocal microscope studies, including using neuronal and glial markers, suggested that differentiation toward a neuronal phenotype was decreased in diabetic animals and was reversed by the antidepressant treatment. In addition, the loss of hilar neurons was avoided by fluoxetine treatment. Several reports have demonstrated that high susceptibility to stress and elevated corticosterone levels are detrimental to neurogenesis and contribute to neuronal loss. These features are common in some types of depression, diabetes, and aging processes, suggesting they participate in the reported hippocampal abnormalities present in these conditions.

Key words: **type 1 diabetes; hippocampus; dentate gyrus; neurogenesis; fluoxetine**

Brain Complications Associated with Diabetes

Diabetes mellitus is one of the most common metabolic diseases in humans. Type 2 diabetes (T2D), mediated by insulin resistance, is much more frequent than type 1 (T1D), which is caused by insulin deficiency. A result in part to changes in nutritional habits and life style, the incidence of both types of diabetes is increasing throughout the world. Both types have significant short- and long-term consequences. The end organs predominantly damaged by the disease are the kidney, retina, peripheral nervous system, and small and large blood vessels. The central nervous system (CNS) has recently been included among the systems affected by acute and chronic diabetes-associated effects. In this regard, neuroglycopenia and accelerated aging

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could be considered among metabolic complications, while stroke and microangiopathy are linked to vascular effects. Brain damage, neurological deficit, and high risk of depression and dementia are some of the related consequences.[1,2]

Aiming to define the status of mild to moderate cognitive impairment in diabetic patients, Mijnhout and co-workers have recently proposed the term "diabetes-associated cognitive decline."[2] In experimental models of this disease, several studies, including ours, agree with the hypothesis of a marked impact of diabetes on the CNS. Working with T1D models, different authors have suggested various mechanisms that operate in diabetic alterations.[3] These include intracellular calcium toxicity, excitotoxic cellular damage associated with excessive glutamate,[4–6] hippocampal astrogliosis, abnormal neural activation,[7,8] and impairment in spatial learning ability.[9–11] Remarkably, the limbic system, including the hippocampus and associated functions, seems to be highly vulnerable to the effects of uncontrolled diabetes.

Hippocampal Neurogenesis Is Impaired in Mice Models of T1D

Adult neurogenesis is the process of generating functionally integrated neurons in two discrete brain regions: the subventricular zone and the dentate gyrus (DG). During the generation of new neurons, some steps are clearly identified in the DG: proliferation, migration through the granular cell layer, maturation, and functional integration into neuronal circuits. The newly generated hippocampal cells have been implicated in learning and memory processes.[12] Several factors and conditions can modulate this event, including gender, hormones, environment, age, early experiences, and physical and mental activity.[13–20] In addition, some pathological conditions can affect the production of new cells in the DG; brain inflammation is linked to a reduced rate of proliferation while after ischemia, trauma, or seizures a transient increase can be observed.[21]

Using 5-bromo-2′-deoxyuridine (BrdU) detection and specific neural markers, we observed a strong reduction in neurogenesis rate in experimental models of T1D. In diabetic mice induced by streptozotocin (STZ) treatment, which is a well-recognized and characterized pharmacological model, a significant decrease in the number of proliferating cells was clear in both neurogenic areas.[22,23] Remarkably, estradiol treatment was able to restore cell proliferation to normal levels.[23]

However, not only cell proliferation was reduced in STZ-treated mice. We showed a decrease in neuronal differentiation of newborn cells in the DG after administration of BrdU in a special protocol before killing the diabetic animals. The phenotype of these BrdU-positive (BrdU+) cells was corroborated by colocalization with immature neuronal markers, such as β-III tubulin/Tuj-1.[24]

The number of hilar neurons, a heterogeneous population of interneurons especially sensitive to brain damage, was also affected by diabetes. Compared with control mice treated with vehicle, the hilus of STZ-treated animals exhibited fewer neurons stained by the Nissl technique.[24]

In separate studies, we obtained consistent results in nonobese diabetic mice (NOD), a spontaneous T1D model that progressively develops the disease. Compared with two control strains (C57BL/6 and BALB/c), the NOD mice showed a reduced hippocampal cell proliferation at three different ages (5-, 8-, and 12-weeks old). Interestingly, at 5 and 8 weeks of age the NOD mice were still not diabetic, suggesting that potential brain alterations could be present even before overt hyperglycemia.[25] When we explored the survival of the newly generated neurons in the DG, we saw an important decline in NOD mice regardless of diabetic condition compared to control strains, but again the reduction was greater in NOD diabetic rodents that exhibited hyperglycemia.

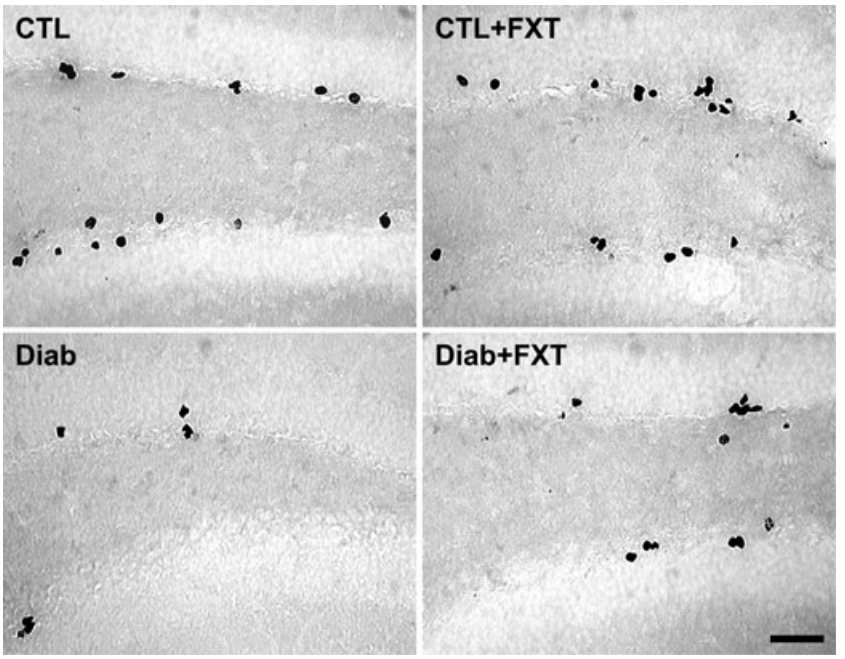

Figure 1. Representative microphotographs corresponding to the cell proliferation protocol of bromodeoxyuridine (BrdU) detection in the dentate gyrus. BrdU-positive cells exhibit darkly stained nuclei and are often distributed in clusters near the subgranular zone. Groups are as follows: vehicle-treated controls (CTL), fluoxetine-treated controls (CTL+FXT), vehicle-treated diabetic mice (Diab), and fluoxetine-treated diabetic mice (Diab+FXT). Note the reduction in the number of BrdU-immunopositive cells in the Diab group compared to the other three groups. Scale bar corresponds to 100 μm.

Antidepressant Treatment Is Able to Recover Reduced DG Neurogenesis in STZ Diabetic Mice

Neurogenesis can be regulated by multiple factors. Stress and some affective-related disorders, such as depression, were associated with high plasma glucocorticoids together with a reduced ability for hippocampal production of new neurons.[15,26,27] Antidepressant treatment was effective in restoring this capability.[27–30]

On the other hand, diabetic subjects present a high prevalence of depression[31–33] and exhibit changes in the serotoninergic system, a feature also found in rodents.[34]

We treated STZ-induced diabetic mice with fluoxetine (a serotonin reuptake inhibitor, 10 mg/kg body weight) over a 10-day period, starting the antidepressant therapy 10 days after diabetes induction, and studied hippocampal cell proliferation and differentiation. The number of BrdU+ cells in the DG significantly increased in diabetic animals after fluoxetine treatment, reaching a rate of proliferation similar to that found in control mice. Interestingly, the experimental control group treated with fluoxetine did not show a significant difference compared to vehicle-treated controls (Fig. 1).

The newly generated cells differentiate mostly into neurons and, to a lesser degree, into glial cells. We performed co-localization studies with neural (Tuj-1) and glial (GFAP) markers in order to establish whether the final phenotype of newborn cells was affected by diabetes. Cellular differentiation was analyzed in fluoxetine-treated diabetic mice injected with BrdU for 7 consecutive days before killing. The proportion of BrdU+ cells also expressing Tuj-1 marker in the DG was increased in diabetic animals under antidepressant treatment compared to

STZ-treated mice treated with vehicle, and it was similar to controls.[24] Our results strongly suggest that not only the number of newborn cells decreased with diabetes but also a decline in the proportion of new cells differentiating into neurons occurred. Fluoxetine administration was able to reverse this situation. The rate of differentiation into GFAP-positive cells was not altered by the diabetic status and was not affected by the antidepressant treatment.[24] Of particular note is that the number of neurons stained with cresyl violet in the hilus of the DG in diabetic mice increased after antidepressant treatment, showing that fluoxetine could be involved in the rescue of these mature neurons.

Concluding Remarks

The hippocampus appears extremely sensitive to the deleterious effects of diabetes. Several reports showed hippocampal alterations in both patients and experimental models. We demonstrated that the diabetic status induced in mice by STZ negatively influences the production of new neurons in both neurogenic brain areas. In the same model, we found a marked reduction in the number of hilar neurons. In the DG of NOD mice, we corroborated a low rate of proliferation that was also, surprisingly, found at prediabetic and diabetic states compared with age-matched control strains. These and other findings are characteristic of an aged or damaged brain and could strongly suggest an accelerated aging process associated with the disease. This finding is in line with those of previously mentioned reports and supports the idea of a diabetic encephalopathy[35] and the more recent concept of diabetes-associated cognitive decline.[36] Interestingly, T2D and obesity could share some brain abnormalities where neuronal systems regulating energy intake and energy expenditure seem to be implicated.[37]

Fluoxetine is a serotonin reuptake inhibitor commonly used with remarkable results in the treatment of depression. In our study we found that fluoxetine treatment during a relatively short period (10 days) was able to prevent some changes in the DG of diabetic mice. Antidepressant treatment effectively increased the proliferation of new cells and the neuronal differentiation of these newborn cells in the DG of STZ-treated mice. In this way, fluoxetine showed efficacy in preventing the loss of hilar density, a neural population especially sensitive to cerebral insult. The lack of effect of fluoxetine treatment on control mice suggests the participation of a mechanism only active during some specific brain alterations or permissive situations.

Several authors have postulated a central role of stress or depression in the atrophy and cell loss in limbic structures associated with higher brain functions.[15,28,38] Reduced birth of new neurons in the adult brain can contribute to this atrophy and/or to a deprived neuronal plasticity, as was observed in experimental models. Diabetes condition is linked to an augmented vulnerability to stress in correlation with a hyperactivity of the HPA axis,[39–42] and this feature, together with high glucocorticoid plasmatic levels, can be intimately involved in adult neurogenesis alterations. Stranahan *et al.* attributed a manifest role to glucocorticoids in the cognitive impairment observed in T1D models,[43] while Revsin *et al.* demonstrated that corticosterone secretion was significantly augmented immediately after diabetes induction in the STZ model but adrenocorticotropic hormone levels were below control levels.[44]

In conclusion, our data have contributed to a better understanding of brain complications associated with a metabolic disease, such as diabetes, and some of these complications are common to depression and accelerated aging. The effectiveness of fluoxetine treatment demonstrated that some of these alterations are not permanent and can be prevented by pharmacological treatment. Further studies are needed to explore the mechanisms and other potential factors involved with the effects of metabolic diseases.

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Conflicts of Interest

The authors declare no conflicts of interest.

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