PERSPECTIVES

Prediabetes and type 2 diabetes implication in central proliferation and neurogenesis

Type 2 diabetes (T2D) is an important risk factor for developing dementias, including Alzheimer's disease (AD). Hyperinsulinemia and glucose intolerance, as features of T2D, might increase the neurodegeneration process, synaptic loss and brain atrophy, leading to cognitive impairment observed in AD. Also, adult neurogenesis seems to be impaired in AD models. Therefore, we have studied morphological alterations, cell proliferation and neurogenesis in the central nervous system (CNS) from a classical model of T2D, the db/db mouse, and in a prediabetes insulin-resistant model, obtained after long-term high fat diet (HFD) administration to C57Bl/6 mice. Db/db mice showed an age-dependent cortical and hippocampal atrophy, whereas in HFD mice cortex and hippocampus were preserved. Neurogenesis and cell proliferation were increased in young db/db mice, when compared with control mice, whereas no differences were detected in the prediabetic model. We also detected significant correlations between metabolic parameters and central atrophy, altered proliferation and neurogenesis in the central nervous system. Altogether these data support that glycaemia control in elderly patients, could help to control central alterations and improve dementia prognosis.

Life expectancy is rapidly increasing in recent years, and therefore the incidence of pathologies associated with ageing are secondarily raising. In this sense, AD is the most common cause of dementia among elderly people. AD is characterized by the presence of senile plaques, neurofibrillary tangles, neuronal loss and cognitive degeneration. On the other hand, ageing is also a main risk factor for developing T2D and increasing evidence supports a cross-talk between T2D and AD (for review, see Craft et al., 2013). Hyperinsulinemia and hyperglycaemia could participate in neurodegenerative disorders and synaptic loss that underlies learning and memory alterations. It has also been reported that neurogenesis is affected in AD models (for review, see Varela-Nallar et al., 2010) as an added limitation to counterbalance neuronal loss, since neurogenesis is one mechanism to replace neurons in the CNS through the activation of stem cells and neuronal progenitor cells. Adult neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricles, which supplies new interneurons to the olfactory bulb in rodent and to the striatum, and the dentate gyrus of the hippocampus in the human brain (Lang et al., 2009; Ernst et al., 2014). To a lesser extend the cortex also presents some neuroregenerative capacity and neurogenesis has also been described in the hypothalamic region.

T2D has been shown to interfere with adult neurogenesis in diabetic murine models as well as limit long term-potentiation and memory (Bruel-Jungerman et al., 2006). Also, previous studies have shown that pituitary adenylate cyclase-activating polypeptide counteracts the impaired adult neural stem cell viability induced by high-fat milieu and therefore receptor agonists may have a potential role in the treatment of neurological complications in obesity and diabetes (Mansouri et al., 2012). Therefore it is feasible that altered central proliferation and neurogenesis in relevant regions for learning and memory, as the cortex and the hippocampus, may underlie observed learning and memory alterations in T2D, and it remains possible that this effect is worsened with ageing, as the disease progresses.

Prediabetic and T2D diabetes mice: metabolic considerations: A great deal of studies have previously described different prediabetic and diabetic models, with significant metabolic alterations. In order to characterize and compare central proliferation and neurogenesis both possibilities were selected (Ramos-Rodriguez et al., 2014). We induced prediabetes to C57Bl/6 mice (Harlan Laboratories, Boxmeer, The Netherlands) as previously described (Ramos-Rodriguez et al., 2013) by chronic administration of a high fat diet (HFD) (60% kcal from fat, Open Source, New Brunswick, NJ, USA) for 18 weeks. HFD feeding started when mice were 8 weeks old and ended at the age of 26 weeks. Control mice for this group, were age-matched C57Bl/6 mice receiving regular diet from our animal facility: SAFE A04 (Augy, France). We also selected a classical T2D model, widely used in the last 5 decades, as it is the db/db mouse. In the diabetic db/db mouse (leptin receptor KO mouse) an *RsaI* site by the *Leprdb* mutation is introduced in the leptin receptor gene (Jimenez-Palomares et al., 2012) resulting in excessive food consumption, precocious and progressive increase in body weight, hyperglycaemia and hyperinsulinemia. C57BlKsJ heterozygous db/+ mice were purchased from Harlan Laboratories (Boxmeer, The Netherlands). Wt, db/db and db/+ mice were generated from crosses between heterozygous db/+ mice. These animals received regular chow and were aged up to 4, 14 and 26 weeks of age. Since heterozygous (db/+) mice do not show specific phenotype (Jimenez-Palomares et al., 2012), Wt and db/+ mice were included in the control group.

Long-term HFD induced a prediabetic state characterized by high insulin levels (> 9 ng/mL), accompanied by increased body weight and an slight increase of glucose levels (under 150 mg/dL), well under 300 mg/dL, considered limit for the diabetic process (Ramos-Rodriguez et al., 2013, 2014). As it could be expected and it has been previously reported, db/db mice presented a progressive increase of body weight and insulin levels from 4 to 26 weeks of age. Also, glucose levels were significantly increased by 14 weeks of age and surpassed the 300 mg/dL limit for definition of T2D (Ramos-Rodriguez et al., 2013, 2014).

Prediabetic and T2D diabetes mice: central atrophy: Histomorphological assessment of brain morphology revealed that prediabetic HFD-treated mice presented similar brain weights as well as cortical and hippocampal areas (Ramos-Rodriguez et al., 2013) and thickness (Ramos-Rodriguez et al., 2014) when compared to untreated mice. These data are in accordance with previous studies on HFD-treated mice with preserved hippocampal synaptic function and long-term potentiation (Mielke et al., 2006). A similar profile was observed in prediabetic db/db mice, since no signifcant differences were observed at 4 weeks of age, when T2D has not debuted yet. As disease progressed we detected a reduction of brain weight that was evident by 14 weeks of age, and significantly worsened by 26 weeks of age, affecting the cortex primarily and the hippocampus only at later stages (Ramos-Rodriguez et al., 2013, 2014). These observations are in accordance with previous MRI studies where cortical volume and thickness are reduced in T2D patients (Brundel et al., 2010). Moreover, we detected that altered metabolic parameters were negatively correlated with central atrophy, and therefore increased body weight, glucose and insulin levels can be considered good predictors of central abnormalities observed.

Prediabetic and T2D diabetes mice: central proliferation and neurogenesis: Cell proliferation, by BrdU immunostaining, and neurogenesis, by doblecortin immunostaining were performed as previously described (Ramos-Rodriguez et al., 2014). Briefly, sections were washed in 0.1 M PBS and incubated in citrate buffer and formamide (1:1) for 2 hours at 65°C followed by 2 N hydrochloric acid incubation for 30 minutes at 37°C for antigen retrieval. After washing in 25 mM borate buffer (pH = 8.4), sections were blocked in 0.1% triton-X in 2.5% BSA (Sigma, Or, USA) and 0.25% sodium azide for 1 hours at room temperature. Primary antibodies (Monoclonal Mouse anti-BrdU 1:100, Dako, Barcelona, Spain and polyclonal IgG Goat anti-DCX 1:400, Santa Cruz Biotechnology, Santa Cruz, CA, USA) were incubated overnight at 4°C. Sections were incubated with secondary antibodies AF594 and AF488 (1:1,000, Invitrogen, Carlsbad, CA, USA). Acquired images were analyzed using Image J software in relevant neurogenic niches as the SVZ and the hippocampus, as well as in the cortex due to its implication in learning and memory. Cell proliferation and neurogenesis was increased up to 14 weeks of age in db/db mice in the most relevant neuroregenerative areas (SVZ and hippocampus) whereas in the cortex this effect was only detectable at early states (4 weeks old), when T2D is not established yet. On the other hand previous studies have reported impaired adult neurogenesis in different diabetic models (Stranahan et al., 2008), and this effect seems to be mediated by corticosterone. Heterozygous db mice do not present diabetic phenotype and therefore they were analyzed with wildtype mice as previously described (Ramos-Rodriguez et al., 2013, 2014), although we can not exclude that different outcomes could be observed in individual groups. No significant differences were observed in case of HFD-treated mice, suggesting that increased glycaemia, rather than hyperinsulinemia might be the major player of this effect, since insulin levels are more severely increased in HFD-treated mice. Cell proliferation occurs in response to an insult and it seems to be impaird with ageing (Varela-Nallar et al., 2010), as we observed in control mice (Ramos-Rodriguez et al., 2014). It is feasible that db/db mice can response at early stages, whereas further impairment, as disease progressed, may preclude the ability of the central nervous system to regenerate, as in 26 weeks old db/db mice.

We can not obviate that the db/db model is a very severe, irreversible model of T2D and the fact that leptin receptors are not functional may preclude other relevant leptin functions, apart from increasing food intake. In this sense, leptin has been implicated in neuronal signaling, learning and memory or long-term potentiation and therefore it remains possible that our observations would be at least partially, due to the lack of the leptin receptor. However the fact that both, brain atrophy as well as proliferation and neurogenesis are affected in an age-dependent manner supports the idea that observed changes are due to the diabetic process, since leptin signalling abnormalities are present from the very beginning in db/db mice. Also, metabolic parameters (body weight, glucose and insulin levels) were negatively correlated with the number of BrdU-positive cells, while there was a positive correlation with the neuroregenerative process, suggesting that where the system cellular production is impaired, the system tries to compensate the generation of new neurons. Altogether, these data suggest that controlling metabolic alterations in T2D, as those observed in db/db mice, could control central complications and improve dementia prognosis.

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