Differential central pathology and cognitive impairment in pre-diabetic and diabetic mice

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Summary   Although age remains the main risk factor to suffer Alzheimer's disease (AD) and vascular dementia (VD), type 2 diabetes (T2D) has turned up as a relevant risk factor for dementia. However, the ultimate underlying mechanisms for this association remain unclear. In the present study we analyzed central nervous system (CNS) morphological and functional consequences of long-term insulin resistance and T2D in db/db mice (leptin receptor KO mice). We also included C57Bl6 mice fed with high fat diet (HFD) and a third group of C57Bl6 streptozotocin (STZ) treated mice. Db/db mice exhibited pathological characteristics that mimic both AD and VD, including age dependent cognitive deterioration, brain atrophy, increased spontaneous hemorrhages and tau phosphorylation, affecting the cortex preferentially. A similar profile was observed in STZ-induced diabetic mice. Moreover metabolic parameters, such as body weight, glucose and insulin levels are good predictors of many of these alterations in db/db mice. In addition, in HFD-induced hyperinsulinemia in C57Bl6 mice, we only observed mild CNS alterations, suggesting that central nervous system dysfunction is associated with well established T2D. Altogether our results suggest that T2D may promote many of the pathological and behavioral alterations observed in dementia, supporting that interventions devoted to control glucose homeostasis could improve dementia progress and prognosis.
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1. Introduction

Progressive increase of life expectancy is secondarily contributing to a significant increase in aging associated pathologies, among which Alzheimer's disease (AD) and vascular dementia (VD) are of special relevance, since these are the most common causes of dementia in elderly people. The most prominent neuropathological features of AD are the presence of senile plaques, composed of amyloid-β peptides, neurofibrillary tangles, primarily composed of abnormally phosphorylated tau, and synaptic loss and brain atrophy (Hyman and Gomez-Isla, 1997). VD is a heterogeneous pathology that ranges from multiple microinfarcts or small vessel ischemic disease, to vascular damage (Craft, 2009). The picture gets more complicated since borderlines between AD and VD are often blurry, and in many patients markers of vascular injury coexist with traditional AD hallmarks (Craft, 2009). On the other hand, the underlying mechanisms are not completely understood and although age remains the main risk factor to suffer dementia, there are other factors that may predispose to suffer AD and VD. In this sense, type 2 diabetes mellitus (T2D) might also play a relevant role in the onset and development of dementia. T2D is a metabolic disorder primarily characterized by insulin resistance and hyperglycemia, and as T2D evolves, other associated pathologies appear, including micro and macrovascular complications and peripheral neuropathy (Kim and Feldman, 2012). Moreover clinical studies suggest that T2D might be a risk factor to suffer AD and VD (Ott et al., 1996; Luchsinger et al., 2004; Plastino et al., 2010).

The close relationship between T2D and dementia has lead to many recent revisions of the field (Kuljis and Salkovic-Petrisic, 2011; de la Monte, 2012) as well as in epidemiological studies (Ott et al., 1996; Craft et al., 2003; Schrijvers et al., 2010; Chen et al., 2011). Moreover a recent study has related specific polymorphism of AMP-activated protein kinase concomitantly to dementia and diabetes (Kim et al., 2012). This relationship has even lead to the identification of a new complex syndrome: type 3 diabetes (de la Monte et al., 2006). However there are still limited studies focusing on the underlying mechanisms (Ho et al., 2004; Bomfim et al., 2012), and only recently animal models harboring both T2D and AD have been developed (Takeda et al., 2010; Hiltunen et al., 2012; Jimenez-Palomares et al., 2012). The diabetic db/db mouse (leptin receptor KO mouse) (Hummel et al., 1966) is a useful model of T2D that results in excessive food consumption, precocious and progressive increase in body weight, hyperglycemia and hyperinsulinemia. Although db/db mice have been profusely used as metabolic syndrome and T2D model, central nervous system (CNS) complications as well as learning and memory alterations derived from diabetes and associated comorbidities observed in this model such as obesity or dyslipidemia, have only been partially characterized in this mouse model. On the other hand whether severe diabetes or just early hyperinsulinemia can induce CNS alterations remains controversial and observations seem to depend on the experimental approach to induce hyperinsulinemia and the specific tests performed afterwards (Arvanitidis et al., 2009; Camargo et al., 2012; Heyward et al., 2012).

The aim of our study was to analyze AD- and VD-related features in the CNS of db/db mice, characterized by long-term insulin resistance and diabetes, as well as associated comorbidities, and secondarily, in a diet-induced model of insulin resistance and hyperinsulinemia. db/db mice exhibited pathological characteristics that mimic both AD and VD, including age-dependent cognitive deterioration as well as brain atrophy, increased presence of spontaneous hemorrhages and increased tau phosphorylation. These alterations preferentially affected cortical regions and were of special relevance once the diabetic process has been established, since a high fat diet (HFD)-induced incipient hyperinsulinemia in C57Bl6 mice only produced mild CNS alterations. Interestingly, metabolic parameters measured in db/db mice, including glucose and insulin levels turned up to be reliable predictors of both, memory impairment as well as cortical atrophy and presence of spontaneous hemorrhages.

2. Materials and methods

2.1. Animals

The mouse model of obesity and type 2 diabetes used in this study is the db/db mouse. The introduction of an *Rsa*I site by the *Lepr*db mutation in the leptin receptor gene was detected by 126 PCR as previously described (Jimenez-Palomares et al., 2012). C57BL/KsJ 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. Animals were aged up to 4, 14 and 26 weeks of age and 7−16 animals/group were included in the studies. Since heterozygous (db/+) mice do not show specific phenotype (Jimenez-Palomares et al., 2012), WT and db/+ mice were included in the control group.

In order to study the possible effect of a pre-diabetic state we also studied a hyperinsulinemic pre-diabetic animal model by including age-matched C57Bl6 mice (Harlam, Boxmeer, Holanda) fed a high fat diet (HFD) (60% kcal from fat, OpenSource, 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 received regular diet from our animal facility: SAFE A04 (Augy, France). A second set of 16 weeks old C57Bl6 mice (n = 5/group) were also included to study possible central alterations as a consequence of diabetes without interfering leptin signaling. As previously described mice were i.p. injected with streptozotocin (STZ) (50 mg/kg) for 5 consecutive days (Shanab et al., 2012) and animals were aged up to 26 weeks of age.

All experimental procedures were approved by the Animal Care and Use Committee of the University of Cadiz, in accordance with the Guidelines for Care and Use of experimental animals (European Commission Directive 86/609/CEE and Spanish Royal Decree 1201/2005).

2.2. Metabolic determinations

Body weight, postprandial blood glucose and insulin levels were determined immediately before sacrifice at all ages under studies (4, 14 and 26 weeks) in db/db mice, as previously described (Jimenez-Palomares et al., 2012). Body weight, postprandial glucose and insulin levels were also determined in C57Bl6 mice at 8 weeks of age (before commencing HFD), at