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SPATIAL MEMORY IMPAIRMENTS IN A PREDIABETIC RAT MODEL

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Abstract—Diabetes is associated with an increased risk for brain disorders, namely cognitive impairments associated with hippocampal dysfunction underlying diabetic encephalopathy. However, the impact of a prediabetic state on cognitive function is unknown. Therefore, we now investigated whether spatial learning and memory deficits and the underlying hippocampal dysfunction were already present in a prediabetic animal model. Adult Wistar rats drinking high-sucrose (HSu) diet (35% sucrose solution during 9 weeks) were compared to controls' drinking water. HSu rats exhibited fasting normoglycemia accompanied by hyperinsulinemia and hypertriglyceridemia in the fed state, and insulin resistance with impaired glucose tolerance confirming them as a prediabetic rodent model. HSu rats displayed a poorer performance in hippocampal-dependent short- and long-term spatial memory performance, assessed with the modified Y-maze and Morris water maze tasks, respectively; this was accompanied by a reduction of insulin receptor-β density with normal levels of insulin receptor substrate-1 pSer636/639, and decreased hippocampal glucocorticoid receptor levels without changes of the plasma corticosterone levels. Importantly, HSu animals exhibited increased hippocampal levels of AMPA and NMDA receptor subunits GluA1 and GLUN1, respectively, whereas the levels of protein markers related to nerve terminals (synaptophysin) and oxidative stress/inflammation (HNE, RAGE, TNF-α) remained unaltered. These findings indicate that 9 weeks of sucrose consumption resulted in a metabolic condition suggestive of a prediabetic state, which translated into short- and long-term spatial memory deficits accompanied by alterations in hippocampal glutamatergic neurotransmission and abnormal glucocorticoid signaling.

Key words: diabetic encephalopathy, high-sucrose diet, prediabetes, hippocampus, memory.

1. INTRODUCTION

The development of type 2 diabetes mellitus (T2DM) is associated with an increased risk for brain disorders (Gold et al., 2007; Bruehl et al., 2009). In particular, a growing body of evidence indicates an increased risk of developing cognitive decline and dementia in a T2DM setting (Roriz-Filho et al., 2009; Xu et al., 2010; Ravona-Springer et al., 2012). T2DM triggers a condition of ''diabetic encephalopathy'' characterized by electrophysiological, structural and neurochemical changes leading to cognitive impairments (Biessels et al., 2002; Ristow, 2004; Mijnhout et al., 2006; Hernández-Fonseca et al., 2009; Sima, 2010). Indeed, memory deficits seem to be the most reliable altered cognitive function in T2DM and seem to have an early onset (Strachan et al., 1997; Winocur et al., 2005; Gold et al., 2007).

These T2DM cognitive deficits have been argued to be due in large part to an impaired central insulin modulation in the hippocampus, which is a critical region for memory processing (McNay and Recknagel, 2011). In fact, adults with newly diagnosed prediabetes or T2DM show an insulin resistance associated with reductions in regional cerebral glucose metabolism and subtle cognitive impairments (Baker et al., 2011). Interestingly, the insulin signaling overlaps with pathways that regulate both synaptic plasticity and memory processes (Kamal et al., 2000; van der Heide et al., 2006; McNay and Recknagel, 2011). Therefore, it is not surprising that insulin has effects on memory storage and synaptic physiology (van der Heide et al., 2006; McNay et al., 2010; Costello et al., 2012).

Accordingly, the preclinical animal studies investigating the relationship between T2DM and cognition have identified mild cognitive deficits (Li et al., 2002; Bélanger et al., 2004; Winocur et al., 2005;

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BBB, blood–brain barrier; Cont, control; EGTA, ethylene glycol tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase ; GFAP, glial fibrillary acidic protein; GR, glucocorticoid receptor; GS, glutamine synthetase; GTT, glucose tolerance test; HFD, high-fat diet; HNE, hydroxynonenal; HOMA, homeostasis model assessment index; HPA, hypothalamic–pituitary–adrenal; HSu, high-sucrose; IGT, impaired glucose tolerance; ITT, insulin tolerance test; NMDAR, N-methyl-d-aspartate receptor; PBS-T, phosphate-buffered saline with 0.1% Tween-20; PSD-95, postsynaptic density protein 95; RAGE, receptor for advanced glycation end products; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TGs, triglycerides; TNF-α, tumor necrosis factor α; ZDF, Zucker Diabetic Fatty.

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Duarte et al., 2012) typified by spatial learning and memory impairments in association with reduced hippocampal long-term potentiation, dendritic spine atrophy, decreased density of glutamatergic terminal markers and abnormal glutamatergic receptors regulation (Trudeau et al., 2004; Duarte et al., 2012). These diabetes-induced changes of hippocampal-dependent memory and plasticity were proposed to result from the over-activation of the abundant hippocampal glucocorticoid receptors (GR) (Sousa and Almeida, 2002; Dorey et al., 2012) by the enhanced levels of corticosterone (Stranahan et al., 2008a) arising from a hyper-activation of the hypothalamic–pituitary–adrenal (HPA) axis that is characteristic of diabetes (Stranahan et al., 2008a; Hwang et al., 2011).

Diabetes is an evolving clinical situation, which is recognized to develop from a situation of metabolic impairment often named as prediabetic state (Tabák et al., 2012). The diagnostic criteria for prediabetes include one or more of the following: impaired fasting glucose [IFG, plasma glucose of 100–125 mg/dL (5.6–6.9 mmol/L)], impaired glucose tolerance [IGT, plasma glucose of 140–199 mg/dL (7.8–11.0 mmol/L) 2 h after an oral load of 75 g dextrose] or hemoglobin A1c 5.7–6.4% (Tabák et al., 2012). Additionally, insulin resistance is already present in the pre-diabetic stage.

However, in contrast to T2DM, it is currently unknown if this condition of mild metabolic dysfunction is already associated with cognitive impairment. Therefore, the present study aimed at developing a model of metabolic dysfunction, based on the consumption of a high-sucrose (HSu) (35% sucrose solution) diet during 9 weeks, to test if pre-diabetic rats displayed learning and memory deficits and an underlying hippocampal dysfunction. We found that metabolic changes suggestive of a pre-diabetic state translated into short- and long-term spatial memory deficits observed, respectively, in the Y-maze and Morris water maze tasks, and alterations on hippocampal glutamate receptors and GR levels.

2. EXPERIMENTAL PROCEDURES

2.1. Animals and experimental procedures

Male Wistar rats (4 months-old) were obtained from Charles River Laboratories (Barcelona, Spain). The animals were housed two per cage, under controlled environmental conditions [12-h light/dark cycle schedule under temperature (22 ± 1 °C) and humidity control]. After an adaptation period of 1 week, rats were randomly divided into two groups (n = 8 animals per group), for a 9-week protocol: (1) control rats continued to drink tap water; (2) HSu-treated rats received 35% sucrose (S0389; Sigma–Aldrich) in the drinking water. All animals were fed standard rat chow, containing 16.1% of protein, 3.1% of lipids, 3.9% of fibers and 5.1% of minerals (AO4 Panlab, Barcelona, Spain) *ad libitum* (with exception in the fasting periods). Food and beverage consumption was monitored for both groups throughout the experiment. The body weight of each animal was recorded weekly during the experimental period. All experiments were approved by the Institutional Animal Care and Use Committee from Faculty of Medicine, Coimbra University, and were performed following the European Community directive (2010/63/EU). All the animals were used for metabolic characterization (see Table 1) and behavioral assays, and within each group, five rats were used for neurochemical analysis.

2.2. Behavioral tasks

After 9 weeks, the short- and long-term spatial memories of control and HSu rats were assessed with a modified Y-maze and a Morris water maze, respectively. After habituation for at least 1 h before the beginning of the tests, behavior was monitored through a video camera positioned above the apparatuses and the images were later analyzed with the ANY Maze video tracking (Stoelting Co., Wood Dale, IL, USA) by an experienced investigator who was unaware of the experimental group being tested.

2.2.1. Water maze task. To evaluate the existence of long-term spatial memory deficits in HSu versus control rats, the animals were submitted to a spatial reference memory version of the water maze using a protocol described by Morris et al. (1982) and previously utilized in our laboratory (Castro et al., 2013). Tests were performed in a circular swimming pool made of black painted fiberglass, with 1.2-m internal diameter and 0.8-m height, and filled with water at 25 °C to a depth of 0.6-m width. The target platform (10 × 10 cm) was made of transparent Plexiglas and was submerged 1–1.5 cm beneath the surface of the water. Starting points for the animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55 × 55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank, and in the standard setting the position of each symbol marked the midpoint of the perimeter of a quadrant (circle = NE quadrant, square = SE quadrant, cross = SW quadrant, and diamond = NW quadrant). The protocol consisted of four training days, four consecutive trials per day, during which the animals were left in the tank facing the wall, then being allowed to swim freely to the submerged platform placed in the center of southwest quadrant of the tank. If the animal did not find the platform during a period of 60 s, it was gently guided to it. The animal was allowed to remain on the platform for 10 s after escaping to it and was then removed from the tank for 20 s before being placed at the next starting point in the tank. The apparatus was located in a room with indirect incandescent illumination. A monitor and a video-recording system were installed in an adjacent room. The experiments were video-taped and the scores for latency of escape from the starting point to the platform and swimming speed were later measured using the ANY-maze® video tracking system. The test session was carried out 24 h later and consisted of a single probe trial where the platform was removed from the pool and each rat was allowed to