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High-fructose intake as risk factor for neurodegeneration: Key role for carboxy methyllysine accumulation in mice hippocampal neurons

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ABSTRACT

Several studies indicate the involvement of advanced glycation end-products (AGEs) in neurodegenerative diseases. Moreover, the rising consumption of fructose in industrialized countries has been related to cognitive impairment, but the impact of fructose-derived AGEs on hippocampus has never been investigated.
The present study aimed to evaluate in the hippocampus of C57Bl/6 mice fed a standard (SD) or a 60% fructose (HFRT) diet for 12 weeks the production of the most studied AGEs, carboxy methyllysine (CML), focusing on the role of the glutathione-dependent enzyme glyoxalase (Glo-1), the main AGEs-detoxifying system, in relation to early signs of neuronal impairment.
HFRT diet evoked CML accumulation in the cell body of pyramidal neurons, followed by RAGE/NFkB signaling activation. A widespread reactive gliosis and altered mitochondrial respiratory complexes activity have been evidenced in HFRT hippocampi, paralleled by oxidative stress increase due to impaired activity of Nrf2 signaling. In addition, a translocation of Glo-1 from axons toward cell body of pyramidal neurons has been observed in HFRT mice, in relation to CML accumulation. Despite increased expression of dimeric Glo-1, its enzymatic activity was not upregulated in HFRT hippocampi, due to reduced glutathione availability, thus failing to prevent CML accumulation. The prevention of CML production by administration of the specific inhibitor pyridoxamine was able to prevent all the fructose-induced hippocampal alterations. In conclusion, a high-fructose consumption, through CML accumulation and Glo-1 impairment, induces in the hippocampus the same molecular and metabolic alterations observed in early phases of neurodegenerative diseases, and can thus represent a risk factor for their onset.

1. Introduction

The worldwide increase in sugars consumption, especially fructose, in recent decades has been related to the epidemic in obesity, dyslipidemia and insulin resistance (Tappy and Le, 2010; Bray and Popkin, 2014; Moore et al., 2014). Less is known about the direct effects of high sugar intake on brain integrity and function, although a relationship between diet-induced insulin resistance and cognitive impairment has been proved (Stranahan et al., 2008). Moreover, previous studies have pointed to the cerebral complications of long-term diabetes, which can lead to a higher risk for the onset of neurodegenerative diseases onset (Verdile et al., 2015; Moreira, 2013). In this contest, several studies have indicated that the linkage between diabetes and susceptibility to neuronal degeneration could be the increased production of advanced glycation end products (AGEs), toxic compounds deriving from the reaction between reducing sugars and proteins (Yang and Song, 2013; Aragno et al., 2005; Allaman et al., 2015). A key role is now consistently recognized for AGEs in Alzheimer's disease and, to a lesser extent, in Parkinson's disease, amyotrophic lateral sclerosis, and prion disease (Li et al., 2012; Salahuddin et al., 2014). In brain of patients the abnormal accumulating proteins linked to these neurodegenerative diseases, such as amyloid beta, tau, prions and transthyretin, were found to be glycated, and this is thought to be associated with the formation of crosslinks that stabilize protein aggregates (Li et al., 2013; Vicente Miranda and Outeiro, 2010). Moreover, protein glycation in brain may be responsible, via the receptor for AGE (RAGE), for an increase in oxidative stress, mitochondrial dysfunction and inflammation through the formation of reactive oxygen species and the induction of NFkB, with subsequent reactive gliosis (Aragno et al., 2005; Li et al., 2013; Wang et al., 2014; Mastrocola et al., 2005; Vicente Miranda and

Abbreviations: AGEs, advanced glycation end products; CML, carboxy methyllysine; DHE, dihydroethidium; GFAP, glial fibrillary acidic protein; Glo-1, glyoxalase-1; GLUT-5, fructose transporter-5; GSH, reduced glutathione; GSSG, oxidized glutathione; Keap1, Kelch-Like ECH-Associated Protein 1; KHK, ketohesokinase; MnSOD, manganese superoxide dismutase; NFkB, nuclear factor-kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; RAGE, receptor for AGE; ROS, reactive oxygen species.

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Outeiro, 2010). In particular, gliosis can be considered as an early protective reaction of astrocytes and microglia to supply energy and sustain neurons metabolism, providing defenses from stresses and stimulating the formation of new synapses. However, if not rapidly resolved, gliosis can exert inhibitory effects in neuroplasticity and regeneration (Pekny et al., 2014).

Accordingly, the inhibition of AGEs production and/or RAGE activation improves brain functions and prevents neuronal degeneration (Salahuddin et al., 2014; Liu et al., 2010). In addition, the positive modulation of AGEs detoxifying systems, such as the glutathione-depending enzyme glyoxalase-1 (Glo-1), has been demonstrated to contrast cognitive decline in a mouse model of Alzheimer's disease (More et al., 2013).

Since fructose has been reported to be a more potent glycating agent than other sugars (Levi and Werman, 1998; Mastrocola et al., 2013; Schalkwijk et al., 2004) and is now widely employed in food and beverages preparation, it has been proposed that a high-fructose intake may play a causal role in cognitive decline and could thus be considered a risk factor for neurodegenerative diseases (Hipkiss, 2014; Hsu et al., 2015; Lakhan and Kirchgessner, 2013; Ross et al., 2009; van der Borght et al., 2011; Yin et al., 2014).

In particular, the hippocampus is the brain region more critical for learning and memory and is intensively investigated in neurodegenerative diseases. The identification of the fructose-induced hippocampal alterations may allow the development of preventive nutritional strategies. The present study was thus conceived to evaluate in the hippocampus of C57Bl/6 mice fed a high-fructose diet the extent of the AGEs production, specifically of carboxy methyllysine (CML), focusing on the expression and activity of the AGEs-detoxifying enzyme Glo-1.

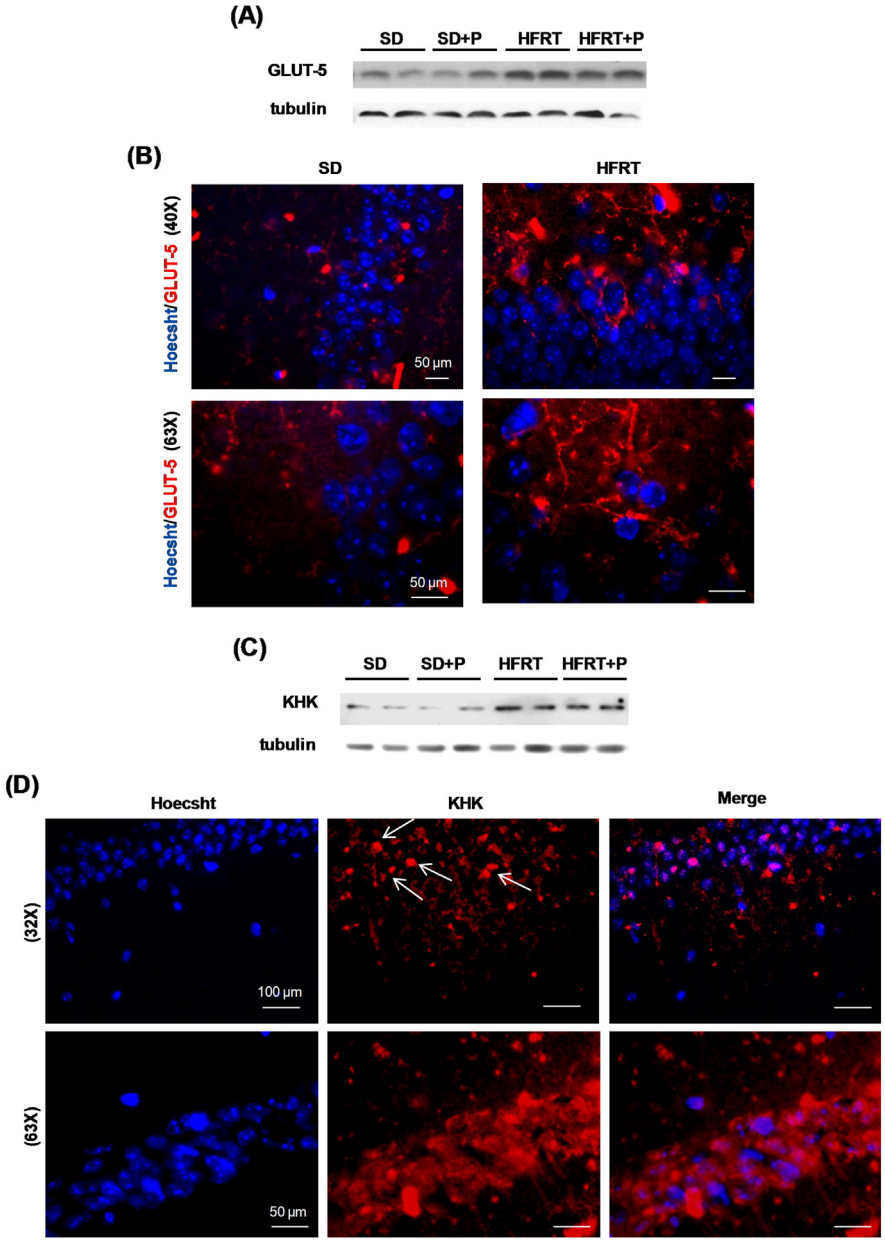

Fig. 1. (A) Representative western blotting analysis for GLUT-5 assessed on hippocampus cytosolic extracts of 6–10 mice per group. The relative tubulin content has been performed to assess correct sample loading. (B) Representative 40/63× magnification photomicrographs of immunofluorescence analysis for GLUT-5 performed on brain sections from SD and HFRT mice, supplemented or not with pyridoxamine. The pyramidal neurons layer of the CA1 region of the hippocampus is evidenced by the nuclear staining with Hoechst dye. (C) Representative western blotting analysis for KHK assessed on hippocampus cytosolic extracts of 6–10 mice per group. (D) Representative 32/63× magnification photomicrographs of immunofluorescence analysis for KHK performed on brain sections from HFRT mice showing expression in the pyramidal neurons layer (white arrows point at the KHK most expressing neurons).