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Impaired cognition and cerebral glucose regulation are associated with astrocyte activation in the parenchyma of metabolically stressed APPswe/PS1dE9 mice

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ABSTRACT

Although metabolic syndrome was suggested to be a risk factor for Alzheimer's disease (AD), the role of metabolic stress in the initiation of AD pathology remains unclear. In this study, metabolic stress was induced by a high-fat diet and low-dose injection of streptozotocin (HFSTZ) before the appearance of senile plaques in APP/PS1 transgenic mice. We found that, HFSTZ treatment exacerbated amyloid beta burden and astrocyte activation in the vicinity of plaques. Moreover, we observed an upregulation of astrocytic S100B expression in the brain parenchyma of HFSTZ-treated APP/PS1 mice concurrent with increased interleukin-6 expression in cerebral microvascular cells. To determine the impact of HFSTZ treatment on brain function, we performed [^{18}F]fludeoxyglucose-positron emission tomography and analyzed nesting behavior. HFSTZ treatment impaired nest construction and cerebral glucose metabolism in several brain regions of APP/PS1 mice during the early stage of AD. These results suggest that HFSTZ-induced peripheral metabolic stress may contribute to vascular inflammation and astrocyte reactivity in the parenchyma and may impair activity of daily living skill and cerebral glucose metabolism in APP/PS1 mice.

1. Introduction

Hyperglycemia, obesity, and insulin resistance are the core features of metabolic syndrome (Samson and Garber, 2014). Impaired metabolic function positively correlates with the development of Alzheimer's disease (AD)–related pathology (De Felice and Ferreira, 2014). The use of transgenic mice has revealed AD pathogenesis, including increased amyloid beta (Aβ) plaque burdens and cerebrovascular inflammation, is accelerated by dietary manipulations (e.g., a high-fat diet [HFD]), knockout of leptin (ob/ob) or mutation of the leptin receptor (db/db), nitrosamine exposure (i.e., administration of streptozotocin) (Jimenez-Palomares et al., 2012; Maesako et al., 2012; Takeda et al., 2010). However, the mechanisms linking metabolic syndrome to AD pathogenesis remain unclear.

Microvascular hemorrhage and hypoperfusion are associated with cognitive impairments in AD patients (Prasad et al., 2014). The accumulation of Aβ due to its overproduction and/or insufficient clearance induces neuroinflammation and neurotoxicity (Heneka et al., 2015). Aβ angiopathy hinders the function of endothelium cells (Stukas et al., 2014), and a defective vascular system reduces the drainage of parenchyma Aβ (Prasad et al., 2014). Therefore, vascular impairment and neurotoxicity resulting from accumulated Aβ constitutes a vicious cycle during the progression of AD pathogenesis.

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Glucose is transported from the blood through endothelium cells by astrocytes (Simpson et al., 2007), which actively modulate neuronal homeostasis and metabolism in the central nervous system (Caruso et al., 2013). Astrocytes extend processes terminating in end feet that encapsulate capillaries to control the blood-brain barrier and molecular exchange between the blood and brain parenchyma (Abbott et al., 2006). Astrocytes sense their surroundings and respond to alterations in their microenvironment (Allaman et al., 2011; Ballabh et al., 2004). In addition, astrocytes near Aβ plaques and neurofibrillary tangles become activated and upregulate the glial fibrillary acidic protein (GFAP) (Thal, 2012).

Over-activated astrocytes are functionally impaired (Tuppo and Arias, 2005). Suppressing astrocyte activation by inhibiting the calcineurin-nuclear factors of activated T cells pathway reduces Aβ burden, suppresses microglial activation, and improves cognitive function in a mouse model of AD (Furman et al., 2012). S100B secreted from astrocytes serves multiple intracellular functions, including stimulating astrocyte proliferation at low concentrations and promoting astrocyte inflammatory activities at high concentrations (Donato et al., 2013). S100B is highly expressed by the reactive astrocytes in close proximity to senile plaques (Mrak and Griffin, 2001). Furthermore, S100B promotes the neuronal production of Aβ, which sets off a feedback loop of S100B and Aβ overproduction and propagation of AD pathogenesis (Li et al., 2011). Moreover, the overexpression of S100B accelerates AD pathology with enhanced astrogliosis and microgliosis in bigenic Tg2576-huS100B mice (Mori et al., 2010).

The aim of the present study was to examine the impact of metabolic syndrome induced before the appearance of AD symptoms on disease pathogenesis. We hypothesized that obesity-induced changes to the vesicular system impair Aβ drainage and transmit an inflammatory signal by activating astrocytes and affecting neuronal function. We examined the Aβ burden, astrocytic expression of S100B and GFAP, vascular expression of interleukin (IL)-6, cerebral glucose metabolism, and cognitive function in APP/PS1 mutant and wild-type (WT) mice.

2. Materials and methods

2.1. Animals

Male APP/PS1 transgenic mice (No. 005864) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) for breeding with female WT C57BL/6J mice. To be brief, the transgenic APP-PS1 (dE9) mouse line expresses human APP with Swedish mutation and mutant human Presinilin 1 (PS1 delta E9) both under the control of the mouse prion protein promoter resulting in abundant amyloid plaques in cortex and hippocampus (Jankowsky et al., 2004). Animals were housed under controlled room temperature (24 ± 1 °C) and humidity (55%–65%) with a 12:12-hour (07:00–19:00) light-dark cycle. Experiments were conducted using male APP/PS1 transgenic mice and their WT littermates. The Institutional Animal Care and Use Committee at the National Research Institute of Chinese Medicine approved the animal protocol (no: 100-A-04 and 102-417-3).

2.2. Induction of metabolic stress with HFSTZ

A high-fat diet and low-dose injection of streptozotocin (HFSTZ) has been shown to induce metabolic stresses such as hyperglycemia, obesity, insulin resistance, and glucose intolerance (Juan et al., 2011). To be brief, starting at the age of 10 weeks, the mice were fed either a normal chow diet (NCD; MF-18, Oriental Yeast Co Ltd, Tokyo, Japan) or an HFD (60% energy from fat; TestDiet, St. Louis, MO, USA) with water *ad libitum*. Mice on an HFD also received intraperitoneal injections of streptozotocin (50 mg/kg) after the mice have been fed with HFD for 2 weeks (HFSTZ group). NCD mice were injected with vehicle (0.1-M citrate buffer, pH 4.5). The HFD continued until mice were killed after 11 or 22 weeks of dietary manipulation.

2.3. Blood glucose analysis

Fasting serum glucose level (mg/dL) was measured using a glucometer (Bioptik Technology, Taipei, Taiwan).

2.4. Measurement of Aβ

At the end of experiments, mice were sacrificed by transcardial perfusion with saline at pH 7.4 and the brain was removed. The cerebral cortex was homogenized in ice-cold homogenization buffer containing 10-mM Tris HCl, 1-mM EDTA, 0.25-M sucrose, and 1-mM phenylmethylsulfonyl fluoride with protease inhibitor cocktail tablets (Roche Diagnostics, Indianapolis, IN, USA). An equal volume of phosphate-buffered saline (PBS) with 1% sodium dodecyl sulfate (SDS) was added to the homogenate. After centrifugation, the supernatant was collected and designated as SDS-soluble Aβ. The insoluble pellet was resuspended in 3-M guanidine HCl for 4 hours and centrifuged, and the supernatant was designated as SDS-insoluble Aβ. The SDS-soluble and SDS-insoluble Aβ samples underwent enzyme-linked immunosorbent assay (ELISA) using Aβ40 and Aβ42 ELISA kits (Life Technologies, Carlsbad, CA, USA). The absorbance was measured at 450 nm using a TECAN plate reader (Sunrise, UK).

2.5. Immunohistochemistry

Mice were perfused with 4% formaldehyde in saline, and brain tissue was cryoprotected. Coronal brain sections (30 μm) were incubated with mouse anti-GFAP (1:500, Millipore, Temecula, CA, USA), rabbit anti-S100B (1:500, Novus, Littleton, CO, USA), or goat anti-IL-6 (1:500, Millipore) at 4 °C overnight. After washing with PBS, brain sections were incubated with Alexa Fluor 594 donkey anti-mouse IgG (1:500, Life Technologies) and Alexa Fluor 633 goat anti-rabbit IgG (1:500, Life Technologies) for 2 hours. Sections were washed with PBS and counterstained with DAPI (4′,6-diamidino-2-phenylindole) (Sigma-Aldrich, St. Louis, MO, USA). Sections were mounted with Aqua Poly/Mount (Polysciences, Inc, Warrington, PA, USA) for microscopic analysis.

2.6. Staining of senile plaques

Coronal brain sections were stained with 0.01% (trans, trans),-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy) styrylbenzene (BSB) as previously described (Skovronsky et al., 2000). To analyze the astrocyte activation in the vicinity of senile plaques, BSB-stained brain sections underwent immunostaining using anti-GFAP and anti-S100B antibodies.

2.7. Quantification of plaque distribution

Fluorescent images of immunohistochemistry and BSB staining were taken using a Zeiss LSM 780 confocal microscope (Carl Zeiss Microscopy, Jena, Germany). Representative images of the cortical distribution of BSB-stained senile plaques were taken at 10× magnification. BSB-stained senile plaques were counted from the images taken at 20× magnification, and the size of senile plaques was measured from images taken at 40× magnification. Eleven z-stacked images spanning 10 μm were compiled with maximum intensity projection. ImageJ software (National