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Effects of diet-induced obesity and voluntary exercise in a tauopathy mouse model: Implications of persistent hyperleptinemia and enhanced astrocytic leptin receptor expression

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ABSTRACT

The number of patients with Alzheimer's disease (AD) is increasing worldwide, and available drugs have shown limited efficacy. Hence, preventive interventions and treatments for presymptomatic AD are currently considered very important. Obesity rates have also been increasing dramatically and it is an independent risk factor of AD. Therefore, for the prevention of AD, it is important to elucidate the pathomechanism between obesity and AD. We generated high calorie diet (HCD)-induced obese tauopathy model mice (PS19), which showed hyperleptinemia but limited insulin resistance. HCD enhanced tau pathology and glial activation. Conversely, voluntary exercise with a running wheel normalized the serum leptin concentration without reducing body weight, and restored the pathological changes induced by HCD. Thus, we speculated that persistent hyperleptinemia played an important role in accelerating pathological changes in PS19 mice. Leptin primarily regulates food intake and body weight via leptin receptor b (LepRb). Interestingly, the nuclear staining for p-STAT3, which was activated by LepRb, was decreased in hippocampal neurons in HCD PS19 mice, indicating leptin resistance. Meanwhile, astroglial activation and the astrocytic expression of a short LepR isoform, LepRa, were enhanced in the hippocampus of HCD PS19 mice. Real-time PCR analysis demonstrated that leptin increased mRNA levels for pro-inflammatory cytokines including IL-1β and TNF-α in primary cultured astrocytes from wild type and LepRb-deficient mice. These observations suggest that persistent hyperleptinemia caused by obesity induces astrocytic activation, astrocytic leptin hypersensitivity with enhanced LepRa expression, and enhanced inflammation, consequently accelerating tau pathology in PS19 mice.

Introduction

The rapid increase in the size of the elderly population has resulted in a dramatic increase in the number of patients with dementia. Of all diseases causing dementia, Alzheimer's disease (AD) is the most prevalent, contributing up to 60–70% of all dementia cases. Current therapies for AD only provide symptomatic relief, either by temporarily improving symptoms above baseline or by delaying cognitive decline. Thus, disease-modifying therapies based on the pathomechanisms of AD are a central focus of AD drug discovery. Although the pathomechanisms of AD are still unclear, β-amyloid protein (Aβ) and tau protein are common targets for disease-modifying therapies. However, recent clinical trials based on the amyloid hypothesis and aiming to reduce the production of Aβ or to remove the accumulated Aβ from the brain have failed to demonstrate significant clinical efficacy, although the hope that these therapies will have some beneficial effects has not been completely abandoned. Additionally, tau-targeting therapies have also failed to yield results for the treatment of AD and related tauopathies, although tau pathology is thought to be more directly related to neurodegeneration and cognitive decline than Aβ pathology (Yoshiyama et al., 2013). Along with these clinical observations, disease-modifying therapies have shown limited efficacy towards improving symptomatic AD patients. Therefore, to prevent the onset of clinical symptoms of AD, researchers have turned their attention to the relationship between lifestyle and AD. Lifestyle-related diseases are potentially preventable, and can be decreased with adequate changes in diet, physical activity, and environment without incurring excessive expense. The common feature at the core of most lifestyle diseases is obesity, rates of which have dramatically increased. Worldwide, age-standardized prevalence of obesity was estimated to be 9.8% in men and 13.8% in woman in 2008, and the highest

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prevalence of obesity was 29.2% in North American men (Finucane et al., 2011). Although it is well-known that obesity is an independent risk factor of AD (Kivipelto et al., 2005; Whitmer et al., 2007; Gustafson et al., 2009; Li et al., 2010; Profenno et al., 2010), obesity induces other AD risk factors. It is therefore difficult to clarify the effects obesity itself and its mechanisms have on AD while excluding other contributing factors and complications related to obesity (such as diabetes mellitus) in human research. Thus, it is important to generate appropriate models to evaluate the pathological relationship between obesity and AD.

High calorie diets (HCD) used in a majority of diet-induced obesity studies usually contain around 550 cal/100 g, and typically induce glucose metabolism abnormalities and insulin resistance (diabetes mellitus). To develop an obese tauopathy mouse model with fewer complicating factors induced by obesity, we used a moderately high calorie diet containing 415 cal/100 g. This allowed us to develop an obese tauopathy mouse model that showed obvious hyperleptinemia but limited insulin resistance. Interestingly, both tau pathology and glial activation were enhanced in these mice, and the expression of certain isoforms of leptin receptor (LepR) was enhanced in astrocytes. Meanwhile, voluntary exercise (EX) with a running wheel reduced hyperleptinemia without altering body weight, and also reduced the tau pathology and glial activation. These findings suggest that the persistent hyperleptinemia might be an important factor that enhances tau pathology.

Methods

Animals

The generation of transgenic (Tg) mice carrying the human tau gene harboring a P301S mutation was previously described by our group (Yoshiyama et al., 2007). Briefly, a cDNA construct of human tau isoform T34 (1N4R) harboring the P301S mutation was cloned into the MoPrP.Xho expression vector containing a mouse prion (MoPrP) promoter (Borchelt et al., 1996) at the XhoI site. A 15-kb NotI fragment containing T34 and the MoPrP promoter together with 39 bp of untranslated sequence was used as the transgene to create tau Tg mice in a B6C3H/F1 background. A stable Tg line (PS19) and non-Tg offspring were identified by PCR analysis of tail genomic DNA.

To reduce potential variations in tau pathology, body weight and metabolism, we used only female mice. Female PS19 mice were exposed to either HCD [15.3% fat (source: beef tallow; composition: saturated, 41%; monosaturated, 44%; polysaturated, 10%: oleic acid, 40%; palmitic acid, 24%; stearic acid, 14%; linoleic acid (n-6), 9%; linolenic acid (n-3), 1%), 415 cal/100 g, Quick Fat (Clea Japan Inc., Japan)] or standard rodent laboratory diet (SD) (4.5 % fat, 330 cal/100 g, Lab Diet 5 L65, Japan SLC, Inc., Japan) from 1.5 to 10 months of age.

The animals in the HCD group were housed in either a regular cage or a cage equipped with a running wheel (CL-4579-2. Clea Japan, Inc., Japan) (from 1.5 to 10 months of age). The average amount of exercise was $9.0 \pm 2.0 \times 10^3$ rounds/day ($\approx 5.6 \pm 1.2$ km/day, mean $\pm$ SD). At 10 months of age, animals were decapitated, and blood (500–700 μl) was collected from the left ventricle of the heart for analysis. Serum samples were stored at −80 °C until measurements. Brain specimens were harvested and hemi-dissected: One hemisphere was microdissected and the other was postfixed in 4% PFA. All tissues used for biochemical analysis were stored at −80 °C, whereas tissue processed for immunohistochemistry was stored at 4 °C. There was no difference in survival among any of the groups. The final numbers of subjects in the SD, HCD, and HCD + EX groups for this study were 13, 12, and 10, respectively.

All experiments were approved by the Committee of Animal Care and Control in Chiba East National Hospital.

Assessment of metabolic changes

Body weight was recorded every 4 weeks throughout the study. Fasting serum insulin and leptin levels were determined using the enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Science, Inc., Japan). Fasting serum total cholesterol level was determined using a colorimetric assay performed with commercial reagents (BioVision, Inc., San Francisco, CA, USA). Fasting serum triglyceride level was determined using Accutrend GCT (Roche Diagnostics, Switzerland). Intraperitoneal glucose tolerance tests (IGTT) were performed in PS19 mice (SD, n = 12; HCD, n = 13; HCD + EX, n = 6) one week before sacrifice. On the night before the IGTTs, food was removed at 6:00 PM, and at 9 AM the next day, and mice were given a single-dose intraperitoneal injection of glucose (2 g/kg body weight) (15 hours fasting). Blood samples were collected from the tail vein immediately before glucose administration and again at 15, 30, 60, and 120 min post-gavage. Blood glucose was assessed using Glutest Ace R (Sanwa Kagaku Kenkyusho Co., Ltd., Japan) following the manufacturer's instructions. Insulin tolerance tests (ITTs) were performed in PS19 mice (SD, n = 10; HCD, n = 12; HCD + EX, n = 6) two weeks before sacrifice. The mice were intraperitoneally injected with insulin (0.75 U/kg diluted in 0.1 ml of 0.9% NaCl). Blood samples were collected from the tail vein immediately before the injection of insulin and again at 15, 30, 45, 60, and 120 min after the insulin injection. Blood glucose was assessed by using Glutest Ace R.

Tau insolubility and phosphorylation states

Proteins were extracted by solubilizing brain tissue in radioimmunoprecipitation assay (RIPA) buffer [50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP40, 5 mM EDTA, and protease inhibitor cocktail (protease inhibitor cocktail set I (Calbiochem, Merck, Germany); final pH 8.0)], using 1 ml/g. Samples were then centrifuged at 40,000 *g* for 40 min at 4 °C. The supernatants were used as RIPA-soluble samples, while the RIPA-insoluble pellets were extracted using 70% formic acid (FA) to recover highly insoluble protein.

Imaging and statistical analyses

Images were captured with a Nikon Eclipse 80i microscope and a Nikon DXM 1200C digital camera, and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD). The results were expressed as the mean ± SE. Statistically significant differences were determined by analysis of variance using SPSS statistical software (SPSS, Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Western blot analysis

Protein concentrations of RIPA-extracted samples were determined using a BCA protein assay kit (Pierce, Rockford, IL), and proteins were resolved by SDS-PAGE, followed by western blotting. Signals were detected by enhanced chemiluminescence (GE Healthcare, WI, USA) according to the manufacturer's instructions. The antibodies and corresponding dilutions used are listed in Table 1.

Primary cortical astrocyte cultures

Enriched cultures of astrocytes were generated using the method developed by McCarthy and de Vellis (1980). First, mixed glia cultures were generated from 1- to 2-day-old mice. Mouse brains were aseptically removed and placed in sterile culture dishes containing Hank's balanced salt solution. The meninges and blood vessels were removed by dissection, and the cerebral cortices (including the hippocampus) were isolated from the brain. The cerebral cortices were mechanically dissociated and suspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 7.5 mM glucose, 4 mM L-glutamine, 1000 U/ml penicillin, and 1 ng/ml streptomycin. Next, cells were centrifuged at 2000 rpm for 5 min, resuspended in 10% DMEM, and placed in 75-cm^2 flask. Cultures were maintained in 10% DMEM at 37 °C in an atmosphere of 5% CO_2. After one week, cells were mechanically dissociated