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Immunohistochemical analysis of tau phosphorylation and astroglial activation with enhanced leptin receptor expression in diet-induced obesity mouse hippocampus

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HIGHLIGHTS

- Diet-induced obesity (DIO) causes tau phosphorylation in wild-type mouse hippocampus.
- DIO enhances astrogliosis, astroglial leptin receptor expression, and mild microgliosis.
- Astroglial leptin receptor may play a role in these pathological processes.
- Voluntary exercise can prevent these DIO-induced pathological changes.

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ABSTRACT

Accumulating evidence indicates that obesity is an independent risk factor for developing Alzheimer disease (AD). Recent studies have shown that diet-induced obesity (DIO) enhances AD-related pathologies in transgenic mouse models of the disease. DIO increases amyloid β (Aβ) deposition in amyloidogenic transgenic mice and enhances tau phosphorylation in tau transgenic mice. However, it remains unclear whether DIO also enhances AD-related pathological processes in wild-type (WT) mice. In this study, we examined the effects of DIO on Aβ and tau pathology in WT mice using immunohistochemistry. In addition, we evaluated the protective effect of voluntary exercise on the DIO-induced pathological changes. DIO caused tau phosphorylation and astroglial activation in the hippocampus in WT mice. Interestingly, these changes were associated with enhanced astrocytic leptin receptor (LepR) expression and mild microgliosis, but not Aβ accumulation. Although phosphorylated tau staining was only observed in the hippocampus, astrogliosis and microgliosis were present in both the amygdala and hippocampus. However, no apparent neuronal loss was observed. Voluntary exercise prevented these DIO-induced pathological changes. Our results demonstrate for the first time that DIO causes tau phosphorylation and that astrocytic LepR might be involved in the pathological process in WT mouse hippocampus. Our findings also suggest that physical exercise is a promising strategy for the prevention of AD in patients with obesity.

1. Introduction

Alzheimer disease (AD) is the most common cause of dementia, with the number of patients increasing steadily worldwide [5]. Accumulating evidence indicates that obesity raises the risk of developing AD, independent of other factors [6,17,20]. Consequently, the risk of developing AD may be decreased by reducing obesity, a preventable disease. However, little is known about the causative link between obesity and AD. AD is pathologically characterized by the extracellular deposition of amyloid β (Aβ) as senile plaques, as well as the intracellular accumulation of

Abbreviations: AD, Alzheimer disease; DIO, diet-induced obesity; Aβ, amyloid β; WT, wild-type; LepR, leptin receptor; GFAP, glial fibrillary acidic protein; LepRs, short form of leptin receptor; LepRl, long form of leptin receptor.

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hyperphosphorylated aggregated tau in the form of neurofibrillary tangles [8]. Recent studies have shown that diet-induced obesity (DIO) enhances AD-related pathology in transgenic mouse models of AD. DIO increases Aβ deposition and memory deficits in APP_{SWE} transgenic mice expressing human APP695 containing the Swedish double mutation (KM670/671NL) [14,15]. DIO also exacerbates tau phosphorylation in the hippocampus and impairs learning ability in tau transgenic mice [12]. These studies clearly show that DIO accelerates AD-associated pathological processes and diminishes cognitive function. Furthermore, APP_{SWE} transgenic mice fed a high-fat diet and given physical exercise show less Aβ deposition than their counterparts fed a high-fat diet without physical exercise [14,15]. These findings indicate that physical exercise can prevent obesity-induced pathological changes in AD mouse models. Additionally, in WT rats, DIO increases glial fibrillary acidic protein (GFAP) expression in the hippocampus [7] and impairs cognitive function [11]. However, it remains unclear whether DIO enhances AD-related pathological processes in WT mice.

The purpose of this study was to examine the effects of DIO on tau pathology using immunohistochemistry in WT mice. We also assessed the protective effect of voluntary exercise on the pathological changes induced by DIO.

2. Material and methods

WT female mice (B6C3H/F1) were divided into high calorie diet (Fat = 15.3%; 4.15 Cal/g, Quick Fat (Clea Japan Inc., Tokyo, Japan))-fed ($n = 21$) and standard diet (Fat = 4.5%; 3.3 Cal/g)-fed (SD, $n = 6$) groups. The high calorie diet group was subdivided into no-exercise (HCD, $n = 17$) and exercise (HCD + Ex, $n = 4$) groups. Mice in the HCD and HCD + Ex groups were fed a high calorie diet, starting at the age of 12 weeks. HCD + Ex group mice were housed in cages equipped with a running wheel (Clea Japan Inc., Japan) for voluntary exercise from the age of 8 weeks. The average amount of exercise was $1.1 \pm 0.4 \times 10^4$ rounds/day ($\approx 7.4 \pm 2.5$ km/day, mean ± SD). Body weight was measured every 4 weeks. Animals were anesthetized by intraperitoneal injection of 1% xylocaine at the age of 40 weeks and decapitated. The body temperature was controlled during the anesthesia using a heating pad. We used PS19 tauopathy model mice, which carry the human tau gene with a P301S mutation [21], as positive controls for evaluating tau pathology. In all experiments, we used only female mice because there was a large difference in body weight between male and female, and the body weight of male mice varied much more widely than that of female mice in a preliminary experiment.

Intraperitoneal glucose tolerance tests (IGTTs) were performed at 40 weeks of age. On the night before the IGTTs, food was removed at 6:00 PM, and the following morning, mice were given a single dose, by intraperitoneal injection, of glucose (2 g/kg body weight). Blood samples were collected from the tail vein immediately before glucose administration and at 15, 30, 60 and 120 min after gavage. Blood glycemic content was assessed using Glutest Ace R (Sanwa Kagaku Kenkyusho Co., Ltd, Nagoya, Japan), following the manufacturer's instructions. For analyses, glucose responses over time were integrated to determine the area-under-the-curve.

Tissue preparation and immunohistochemical analysis were performed as previously described [21]. Briefly, mice were deeply anesthetized and transcardially perfused with 15 ml phosphate-buffered saline (PBS). The brains were removed, immersion-fixed for 24 h in 4% paraformaldehyde/PBS, and stored in 15% sucrose/PBS at 4 °C until use. The brains were cut into 20-μm-thick sections for immunohistochemical analysis. Free-floating sections were immunostained using streptavidin-biotin peroxidase.

The following antibodies were used in this study: mAb AT8 (1:100; Innogenetics, Gent, Belgium), mAb AT180 (1:100; Innogenetics), mAb S422 (1:100; Invitrogen, CA, USA), anti-glial fibrillary acidic protein (1:1000; Dako, CA, USA), anti-Iba-1 (1:1000; Wako, Osaka, Japan), anti-leptin receptor M18 (1:100; Santa Cruz Biotechnology, CA, USA), and anti-leptin receptor C14104 (1:100; Neuromics, MN, USA).

Images were captured with a Nikon eclipse 80i microscope and a Nikon DXM 1200C digital camera. To measure the area occupied by S422-, GFAP-, Iba-1- and M18-positive cells in the hippocampus and amygdala we obtained three images each of the CA1, CA3 and dentate gyrus regions of the hippocampus and five images each of the amygdala for each animal. The quantification of the positively labeled areas was performed using Photoshop (Adobe Systems, CA, USA). The number of neurons and the number of GFAP- and Iba-1 positive cells were measured manually using digital photomicrographs. Quantification results were analyzed using one-way ANOVA, followed by Tukey's post hoc test or Scheffe's post hoc test. Results were expressed as mean ± SD, and differences with $P < 0.05$ were considered significant.

3. Results

To evaluate the effect of diet and exercise, we measured body weight gain every 4 weeks. After 20 weeks of age, the body weight of HCD mice was significantly increased compared with SD mice or HCD + Ex mice (Fig. 1A). To estimate glucose tolerance, we performed IGTTs on the three groups. Although serum glucose at 60 min was slightly elevated in the HCD group compared with the SD or HCD + Ex group, the area-under-the-curve was not significantly different among the three groups (Fig. 1B). These findings suggest that glucose metabolism is not significantly perturbed in HCD mice.

To assess the effect of DIO on the tau phosphorylation, the neuropathological hallmarks of AD, we performed immunohistochemistry using several phosphorylation-specific tau antibodies; S422 (phospho-Ser422), AT-8 (phospho-Ser202 and phospho-Thr205) and AT-180 (phospho-Thr231). Sections from the three different groups in WT mice were not labeled with AT-8 (Fig. 1C) or AT-180 (Fig. 1D). Interestingly, dendrites in the CA1 region and mossy fiber in the CA3 region were slightly stained with S422 in HCD mice (Fig. 1G and H), but not in SD (Fig. 1E and F) or HCD + Ex (Fig. 1I and J) mice. As positive controls, perikarya and neurites in the CA1 of the hippocampus from the 24-week-old PS19 mice were strongly stained with AT-8 (Fig. 1C, inset), AT-180 (Fig. 1D, inset), or S422 (Fig. 1E, inset), respectively. Quantitative analysis revealed that the percentage of the area occupied by S422-positive cells is higher in HCD mice than in SD or HCD + Ex mice (Fig. 1K). We also examined the amygdala and other brain regions, but they were not stained with S422, AT-8 or AT-180. It is known that tau phosphorylation occurs in neurites in early phase [18]. From these results, we conjectured that DIO can at least partially induce tau phosphorylation in the hippocampus in WT mice. Next, to assess neurodegeneration, we counted the number of neurons in the hippocampus. The number of neurons in the hippocampus was not significantly different among the three different groups (data not shown), suggesting that neurodegeneration was not present.

Because astrogliosis parallels the distribution of tau phosphorylation in neurodegenerative diseases [2,19], we performed immunohistochemistry with an antibody to GFAP. The number of GFAP-immunoreactive astrocytes in the CA1 and CA3 regions of the hippocampus was significantly increased (Fig. 2B and E) and some astrocytes displayed hypertrophic changes (Fig. 2B, E, inset) in HCD mice compared with SD (Fig. 2A and D) or HCD + Ex mice (Fig. 2C and F). Similar changes were observed in the amygdala (Fig. 2G–I). Quantitative analysis revealed that the percentage of the area occupied by GFAP-positive cells (Fig. 2P) and the number of astrocytes