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Differential effects of voluntary treadmill exercise and caloric restriction on tau pathogenesis in a mouse model of Alzheimer's disease-like tau pathology fed with Western diet

Maud Gratuze[a,b,*], Jacinthe Julien[b], Françoise Morin[b], André Marette[c], Emmanuel Planel[a,b,*]

[a] Université Laval, Faculté de médecine, Département de Psychiatrie et Neurosciences, Québec, Canada
[b] Centre de Recherche du CHU de Québec, Axe Neurosciences, Québec, Canada
[c] Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec, Canada

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ABSTRACT

Background: Tau is a microtubule-associated protein that becomes pathological when it undergoes hyperphosphorylation and aggregation as seen in Alzheimer's disease (AD). AD is mostly sporadic, with environmental, biological and/or genetic risks factors, interacting together to promote the disease. In the past decade, reports have suggested that obesity in midlife could be one of these risk factors. On the other hand, caloric restriction and physical exercise have been reported to reduce the incidence and outcome of obesity as well as AD.
Methods: We evaluated the impact of voluntary physical exercise and caloric restriction on tau pathology during 2 months in hTau mice under high caloric diet in order to evaluate if these strategies could prevent AD-like pathology in obese conditions.
Results: We found no effects of obesity induced by Western diet on both Tau phosphorylation and aggregation compared to controls. However, exercise reduced tau phosphorylation while caloric restriction exacerbated its aggregation in the brains of obese hTau mice. We then examined the mechanisms underlying changes in tau phosphorylation and aggregation by exploring major tau kinases and phosphatases and key proteins involved in autophagy. However, there were no significant effects of voluntary exercise and caloric restriction on these proteins in hTau mice that could explain our results.
Conclusion: In this study, we report differential effects of voluntary treadmill exercise and caloric restriction on tau pathogenesis in our obese mice, namely beneficial effect of exercise on tau phosphorylation and deleterious effect of caloric restriction on tau aggregation. Our results suggest that lifestyle strategies used to reduce metabolic disorders and AD must be selected and studied carefully to avoid exacerbation of pathologies.

1. Introduction

Tau is a microtubule-associated protein that regulates microtubule stability, neurite outgrowth, and axonal transport (for review (Johnson and Stoothoff, 2004)). Tau can become pathological when it undergoes hyperphosphorylation and aggregation (Grundke-Iqbal et al., 1986). Neurodegenerative diseases with Tau pathology are called Tauopathies, the most well known and widespread being Alzheimer's disease (AD). Tau pathology is a crucial event for this disease since its spreading strongly correlates with cognitive alterations (Arriagada et al., 1992; Bretteville and Planel, 2008; Duff and Planel, 2005).

AD is mostly sporadic (99%), with environmental, biological and/or genetic risks factors, interacting together to promote the disease (Ritchie and Lovestone, 2002). In the past decade, reports have suggested that unhealthy eating habits could be one of these risk factors (2014 Alzheimer's disease facts and figures, 2014). Indeed, epidemiological studies show that metabolic disorders like type 2 diabetes (T2DM) or obesity are associated with a higher risk of AD later in life

Abbreviations: AD, Alzheimer's disease; T2DM, type 2 diabetes; Aβ, amyloid-β peptide; CTL, control; WD, Western diet; ex, voluntary exercise; CR, caloric restriction; HFCS 55, High Fructose Corn Syrup 55; AMPKα, AMP-activated protein kinase α; IGF-1, Insulin-like growth factor-1; CamKII, Ca2 + calmodulin-dependent protein kinase II; JNK, c-Jun N-terminal kinase; GSK-3β, glycogen synthase kinase-3β; ERK, mitogen activated protein kinase/extracellular signal-regulated kinase; cdk5, cyclin-dependent kinase 5; PP, phosphatase; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; LAMP1, Lysosomal-associated membrane protein 1; ATGs, Autophagy proteins; HSP70, 70 kDa heat shock protein; LC3, Microtubule-associated protein 1A/1B-light chain 3; SNAP 25, Synaptosome Associated Protein 25; PSD-95, postsynaptic density protein 95

* Corresponding authors at: CHUL, RC-9800, 2705 Boulevard Laurier, Québec, Canada.
E-mail addresses: maudgratuze@gmail.com (M. Gratuze), jacinthe.julien@crchudequebec.ulaval.ca (J. Julien), Francoise.Morin@crchul.ulaval.ca (F. Morin), andre.marette@criucpq.ulaval.ca (A. Marette), emmanuel@planel.org (E. Planel).

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(Beydoun et al., 2008; Kivipelto et al., 2005; Leibson et al., 1997; Ott et al., 1999; Profenno et al., 2010). Unhealthy diet including overconsumption of fat, sugar and cholesterol, which is better known as Western diet, is a major risk factor for metabolic disorders (Montonen et al., 2005; Newby et al., 2004,2003; Nolan et al., 1995; Schulze et al., 2006; van Dam et al., 2002). On the other hand, caloric restriction and physical exercise have been reported to reduce the incidence and outcome of metabolic disorders as well as AD (Hillman et al., 2008; Larson et al., 2006; Lautenschlager et al., 2008; Scarmeas et al., 2009). Moreover, AD-like transgenic mice exhibit less amyloid burden and tau pathology when subjected to caloric restriction (Halagappa et al., 2007; Mouton et al., 2009; Patel et al., 2005; Wang et al., 2005) or physical exercise (Belarbi et al., 2011; Garcia-Mesa et al., 2011; Liu et al., 2013; Ohia-Nwoko et al., 2014; Tapia-Rojas et al., 2016). While much research has been devoted to understanding the mechanisms by which physical activity or caloric restriction can reduce or prevent Tau and Aβ pathologies, it still remains unknown what kind of strategies targeting metabolic conditions is more effective. In addition, there is only few studies assessing impact both caloric restriction and physical activity on AD hallmarks under high caloric diet in order to evaluate if these strategies could prevent AD emergence in impaired metabolic conditions. Therefore, we compared the impact of voluntary physical exercise and caloric restriction on tau pathology in Western diet fed hTau mice, a humanized tau transgenic mouse. Caloric restriction is usually defined as a decrease in caloric intake compared to the previous diet (whether control diet or hypercaloric diet) in order to reduce body weight without incurring malnutrition or a reduction in essential nutrients. In our protocol, caloric restriction corresponded to a shift from Western diet to control diet. We found no effects of obesity induced by Western diet on both tau phosphorylation and aggregation compared to controls. However, we report differential effects of voluntary treadmill exercise and caloric restriction on tau pathogenesis in our obese mice, namely beneficial effect of exercise on tau phosphorylation and deleterious effect of caloric restriction on tau aggregation. Our results suggest that lifestyle strategies used to reduce metabolic disorders and AD must be selected and studied carefully to avoid exacerbation of pathologies.

2. Methods

2.1. Animals

The hTau mice (Andorfer et al., 2005; Andorfer et al., 2003) were generated by crossing 8c mice that express a tau transgene derived from a human PAC containing the coding sequence, intronic regions, and regulatory regions of the human gene (Duff et al., 2000), with tau knockout mice that have a targeted disruption of exon one of tau (Tucker et al., 2001). They were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) (B6.Cg-Mapttm1 (EGFP)Klt Tg(MAPT) 8cPdav/J) on C57BL/6J background. Mice of either sex were maintained in a temperature-controlled room (~23 °C) with a light/dark cycle of 12/12 h, and experiments were performed during the light period. All animals had access *ad libitum* to pelleted food from Harlan Teklad (Madison, WI, USA) (see Table S1 for exact diet composition) and water *ad libitum* either a standard purified diet (CTL; TD.94045) or a Western diet (WD; 45% of Kcal in Fat, 0.5% of cholesterol (TD.140284)) and 15% of High Fructose Corn Syrup (HFCS 55) in drinking water (Nature's Flavors) starting at 2 months of age during 6 months (Fig. 1). We used HFCS 55 (composition: 55% fructose, 42–44% glucose and 1–3% other sugars/polysaccharides) because of its common use in the food industry (Marriott et al., 2009). This sugar mixture was diluted into drinking water to mimic HFCS 55 consumption by humans (soft drinks and other beverages). To examine the effect of voluntary exercise on hTau mice fed with Western diet (WD + ex group), a running wheel was added in the cage of mice after 4 months of WD. The mice spent 2 months in the exercise condition and under WD. To examine the effect of caloric restriction after WD on hTau mice, WD was replaced with standard purified diet *ad libitum* after 4 months of WD and mice were fed with control diet for 2 months (WD + CR group). Animals were handled according to procedures approved by the Comité de Protection des Animaux du CHU under the guidelines of the Canadian Council on Animal Care.

2.2. Physiological parameters

Fasting blood glucose was measured using a glucometer with reagent strips (ACCU-CHEK® Aviva Nano; Roche Diagnostics GmbH, Mannheim, Germany). Mice were weighed weekly from the beginning of diets until sacrifice. All mice were weighted at sacrifice and the body temperature was monitored using a rectal probe (Thermalert TH-5, Physitemp, Clifton, NJ, USA).

2.3. Protein extraction

Mice were killed by decapitation without anesthesia, as anesthesia can lead to hypothermia-induced tau hyperphosphorylation (Planel et al., 2007). Brains were immediately removed and the tissues dissected on ice, frozen on dry ice, and kept at − 80 °C until they were processed as previously described (Julien et al., 2012). Briefly, dissected brain structures (hippocampus, cortex) were homogenized, without thawing, in 5 times volume/weight of radio immunoprecipitation assay RIPA buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 150 mM NaCl, 0.25% Na-deoxycholate, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, 1 mM PMSF, 10 µl/ml of Proteases Inhibitors Cocktail (P8340, Sigma-Aldrich, St. Louis, MO)), using a mechanical homogenizer (TH, Omni International, Marietta, GA). Samples were then centrifuged for 20 min at 20,000*g* at 4 °C. The supernatant was recovered, diluted in sample buffer (NuPAGE LDS; Invitrogen, Carlsbad, CA) containing 5% of 2-β-mercapto-ethanol, 1 mM Na_3VO_4, 1 mM NaF, 1 mM PMSF, 10 µl/ml of Proteases Inhibitors Cocktail (P8340; Sigma-Aldrich), boiled for 5 min and kept then at − 20 °C.

2.4. Analysis of aggregates and soluble tau

Tau aggregates were extracted according to a protocol previously used to isolate tau aggregates in mouse models of tauopathies (Julien et al., 2012). This procedure uses 1% sarkosyl and derives from a protocol used to isolate tau aggregates from the brains of AD patients (Greenberg and Davies, 1990).

Briefly, the RIPA supernatant was adjusted to 1% sarkosyl (*N*-lauroylsarcosine), incubated for 30 min at room temperature with constant shaking, and centrifuged at 100,000 × *g* for 1 h at 20 °C. The pellet containing sarkosyl-insoluble aggregated (SP fraction) was resuspended and diluted in Sample buffer (NuPAGE LDS) containing 5% of 2-β-mercapto-ethanol, 1 mM Na_3VO_4, 1 mM NaF, 1 mM PMSF, 10 µl/ml of Proteases Inhibitors Cocktail (P8340, Sigma-Aldrich), boiled for 5 min, and kept at − 20 °C.

For heat stable soluble tau, the RIPA supernatant was boiled for 5 min and centrifuged at 20,000 × *g* for 20 min. The supernatant was recovered, diluted in sample buffer (NuPAGE LDS; Invitrogen, Carlsbad, CA) containing 5% of 2-β-mercapto-ethanol, 1 mM Na_3VO_4, 1 mM NaF, 1 mM PMSF, 10 µl/ml of Proteases Inhibitors Cocktail (P8340; Sigma-Aldrich) and boiled for 5 min (Heat stable fraction).

2.5. Western-blot analysis

SDS-PAGE and Western blot analysis were done as previously described (Julien et al., 2012). All antibodies used in this study are listed in Table S2. All antibodies were commercial except for PHF-1 (Otvos et al., 1994), CP13 (Weaver et al., 2000), MC-6 (Jicha et al., 1997) and CP27 (Duff et al., 2000), which were a generous gift from Dr. Peter Davies. Depending on the antibody used, 5–30 µg of brain protein were