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OBESITY, DIABETES, AND LEPTIN RESISTANCE PROMOTE TAU PATHOLOGY IN A MOUSE MODEL OF DISEASE

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Abstract—Obesity and type 2 diabetes mellitus (T2DM) convey an increased risk for developing dementia. The microtubule-associated protein tau is implicated in neurodegenerative disease by undergoing hyperphosphorylation and aggregation, leading to cytotoxicity and neurodegeneration. Enzymes involved in the regulation of tau phosphorylation, such as GSK3β, are tightly associated with pathways found to be dysregulated in T2DM. We have shown previously that leptin-resistant mice, which develop obesity and a diabetic phenotype, display elevated levels of tau phosphorylation. Here we show cells cultured with leptin, an adipokine shown to have neuroprotective effects, reduces tau phosphorylation. To explore how this mechanism works *in vivo* we transduced an existing diabetic mouse line (*Lepr*$^{db/db}$) with a tau mutant (*tau*^{P301L}) via adeno-associated virus (AAV). The resulting phenotype included a striking increase in tau phosphorylation and the number of neurofibrillary tangles (NFTs) found within the hippocampus. We conclude that leptin resistance-induced obesity and diabetes accelerates the development of tau pathology. This model of metabolic dysfunction and tauopathy provides a new system in which to explore the mechanisms underlying the ways in which leptin resistance and diabetes influence development of tau pathology, and may ultimately be related to the development of NFTs.

Key words: tau, leptin, obesity, diabetes, Alzheimer's disease.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) affects over 25 million individuals in the U.S. (Adeghate et al., 2006; Prevention, 2011). T2DM is a metabolic disorder associated with insulin resistance, dysregulated intracellular signaling (elevated glucagon release, failure of glucose receptor recruitment, and reduced lipolysis to name a few), and pancreatic β-cell degeneration in late stages of the disease, which ultimately result in systemic glucose mishandling (Baker et al., 2011; Accardi et al., 2012; Bergman, 2013). Obesity is a common driving factor for the development of T2DM and the two diseases are highly associated, with 85.2% of T2DM patients being classified as overweight or obese (Ford et al., 1997; Resnick et al., 2000; Mokdad et al., 2003; Prevention, 2011). Chronic obesity and T2DM often result in a series of secondary pathologies, including cardiovascular disease, renal dysfunction, and dementias (Rewers et al., 2004; Xu et al., 2009; Seshasai et al., 2011; Association, 2013).

Due to improved therapeutics, individuals with T2DM are living longer and are, therefore, living into the ages where neurodegenerative diseases develop. However, these patients are at a greater risk of developing neurodegenerative disease, such as Alzheimer's disease (AD), mild cognitive impairment, and vascular dementia, than healthy, similarly aged counterparts (Stewart and Liolitsa, 1999; Yaffe et al., 2004; Craft, 2005; Messier, 2005; Profenno et al., 2010; Chen et al., 2012). Though this link is well-established, the underlying mechanisms involved in the development of pathology remain unclear. Previous studies have focused on amyloid or tau accumulation, inflammation, and cerebrovascular disease driven by diabetes and/or obesity (Anguiano et al., 2002; Desai et al., 2014; Ferreira et al., 2014).

Tau binds to microtubules which not only support cellular structure, but also provide a physical pathway for important axonal transporters, such as dynein and kinesin (Weingarten et al., 1975). Tau is regulated by many different kinases and phosphatases which modify its phosphorylation state and microtubule-binding capabilities (Morishima-Kawashima et al., 1995; Augustinack et al., 2002; Cavallini et al., 2013). When tau becomes hyperphosphorylated, it can no longer bind to, and stabilize, microtubules. In addition, hyperphosphorylated tau has a tendency to aggregate, resulting in the formation of higher order structures, such as oligomers, paired helical filaments (PHFs), and neurofibrillary tangles (NFTs) (Augustinack et al., 2002; Iqbal et al., 2005). NFTs are a classic hallmark of tauopathy observed in many

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Abbreviations: AAV1, adeno-associated virus serotype 1; AD, Alzheimer's disease; EDTA, ethylenediaminetetraacetic acid; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; GSK3β, glycogen synthase kinase-3β; GTTs, glucose tolerance tests; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MWM, morris water maze; mTOR, mammalian target of rapamycin; NFT, neurofibrillary tangle; PHFs, paired helical filaments; SDS, sodium dodecyl sulfate; STAT, signal transducer and activator of transcription; T2DM, type-2 diabetes mellitus; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element.

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neurodegenerative diseases, such as AD, frontotemporal dementia (FTD), and may be observed in other neurodegenerative diseases such as Parkinson's disease (Kosik et al., 1986; Wood et al., 1986; Williams, 2006). Though it is unclear whether NFTs directly induce neurodegeneration, their presence is associated with neuronal death (Kril et al., 2002). On the other hand, it has been suggested that NFTs may form as a protective mechanism to counter the effects of oxidative stress or to sequester the cytotoxic oligomeric species of tau (Sayre et al., 2000; Maeda et al., 2006; Lasagna-Reeves et al., 2012).

Multiple studies have demonstrated that tau pathology can be modulated by diabetes or obesity. Streptozotocin-induced diabetes results in tau hyperphosphorylation in mice (Planel et al., 2007). Alterations in tau splice patterns and increases in tau phosphorylation have been observed in rodent models of T2DM (Kim et al., 2009; Jung et al., 2011). Additionally, hyperinsulinemic rats display increases in tau hyperphosphorylation (Freude et al., 2005). These changes to tau regulation are likely due to dysfunction within the myriad of pathways impacted by obesity and diabetes (Virkamaki et al., 1999; Schmelzle et al., 2006; Rains and Jain, 2011). For instance, insulin signaling transiently modulates tau phosphorylation in primary cortical neurons when stimulated with Insulin-like growth factor 1 (Lesort and Johnson, 2000). Collectively, these data suggest that diabetes-associated metabolic dysfunction influences tau phosphorylation, and may promote pathogenesis (Freude et al., 2005).

Insulin signaling is only one of the signaling pathways disrupted in the chronically obese or diabetic state. Leptin is a hormone secreted from adipose tissue that regulates satiety and energy expenditure via the hypothalamus. The leptin receptor is expressed throughout the brain, suggesting it plays additional roles outside hypothalamic regulation (Couce et al., 1997; Shioda et al., 1998; Burguera et al., 2000). Leptin signaling has also been shown to reduce tau phosphorylation (Greco et al., 2008; Marwarha et al., 2010). Individuals who are obese and diabetic often develop resistance to leptin signaling (Campfield et al., 1995; Halaas et al., 1995; Considine et al., 1996; Ostlund et al., 1996). Leptin signaling classically regulates transcription via Janus kinase and signal transducer and activator of transcription (JAK-STAT) pathways to modulate energy metabolism and satiety (Hakansson and Meister, 1998; Elias et al., 1999; Munzberg et al., 2003). However, leptin signaling also involves a variety of other signaling cascades including the PI3K/AKT, MAPK, and mammalian target of rapamycin (mTOR) pathways (Banks et al., 2000; Bjorbaek et al., 2001; Niswender et al., 2001; Zhao et al., 2002; Rahmouni et al., 2003; Cota et al., 2006, 2008). Plasma leptin levels have shown to be inversely correlated with dementia risk, and leptin treatment has been shown to increase cognition and reduce amyloid pathology in transgenic mice (Harvey, 2007; Lieb et al., 2009; Greco et al., 2010). In addition, our lab has demonstrated that leptin reduces β-amyloid production, likely as a result of lower γ-secretase expression (Niedowicz et al., 2013).

Though other studies have examined the effect of diabetes on tau phosphorylation, to our knowledge, no studies have looked directly at the effects of obesity and diabetes on the development of tau pathology, the neurodegenerative driving hallmark. In this study, we transduced leptin-resistant mice ($Lepr^{db/db}$), which develop obesity and diabetes with adeno-associated virus serotype 1 (AAV1) tau^{P301L}. We show that obesity and diabetes promote tau hyperphosphorylation and the accumulation of tau pathology.

EXPERIMENTAL PROCEDURES

Mice

All animal work was approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC) (Protocol Number: 2010-0673), and was performed in accordance with PHS guidelines. All procedures were performed under conditions designed to minimize pain and distress. The University of Kentucky is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved institution, and follows the current version of the Guide for the Care and Use of Laboratory Animals (8th Edition), as adopted by the Office of Laboratory Animal Welfare (OLAW). The work with recombinant virus was approved by the University of Kentucky Institutional Biosafety Committee (B11-1629).

Leptin receptor-deficient mice were purchased from the Jackson Laboratory (B6.BKS(D)-$Lepr^{db}$/J, stock #000697) and were housed two to three mice per cage with *ab libitum* access to food and water and maintained under a 12-h light/dark cycle. All husbandry and treatment procedures were conducted with prior approval of the University of Kentucky's Institutional Animal Care and Use Committee, in accordance with PHS guidelines. Heterozygous males and females were used for breeding (as mice homozygous for the *db* mutation are infertile). The offspring were genotyped by $Lepr^{db}$ single-nucleotide polymorphism-specific qPCR using a Taqman® genotyping kit (Applied Biosystems by Life Technologies; Grand Island, NY, USA) with Quanta Accustart Genotyping Toughmix® (Quanta Biosciences; Gaithersburg, MD, USA). Previous studies using AAV1 vectors to transduce the tau^{P301L} mutation indicated that extensive tangle pathology develops by 6 months of age, therefore we chose 6 months to be our endpoint for these mice (Klein et al., 2004b). Mice were subjected to glucose tolerance tests (GTTs) at 22 weeks and morris water maze (MWM) at 23 weeks of age. Mice were euthanized at 6 months by administration of a lethal dose of Beuthanasia-D (Henry Schein Animal Health; Dublin, OH, USA). Upon euthanasia, brains were collected and divided along the sagittal plane; one half of the brain was drop-fixed in 10% PBS-buffered formalin, and then transferred (after a minimum of 24 h) to phosphate-buffered saline (PBS) with 0.05% NaN_3 for storage. Whole blood was collected in tubes containing EDTA (Starstedt; Newton, NC, USA), centrifuged at 1500×*g* for 10 min, and the plasma collected and frozen for future analysis.