Impaired glucose tolerance precedes neuroanatomical identification of Aβ and hyperphosphorylated tau accumulation in the 3xTg model of Alzheimer’s disease

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Introduction

• Alzheimer’s disease (AD) is a neurodegenerative disease characterized by dementia, deposition of beta-amyloid plaques and neuritic tangles.
• Data suggest that alterations in glucose tolerance and insulin secretion and/or sensitivity are a risk factor for disease progression (Rönnemaa 2007, Li 2007, Rinnmöes 2008). This could be because disruptions in insulin signaling can affect Aβ clearance in AD and thus neuroprotection (De Felice 2009, Xie 2007). Furthermore, hyperglycemia itself can be harmful by inducing oxidative stress (Negre-Salvayre 2009). Thus, alterations in glucose tolerance due to changes in insulin production or in insulin responsiveness/sensitivity may be a driving force for AD pathogenesis.
• In this study we evaluated whether glucose tolerance is altered in a mouse model of AD (3xTg mice). We hypothesized that such changes, if observed, would occur prior to the appearance of CNS pathology or cognitive deficits.

Methods:

Animals: Age matched male 3Tg-AD mice (2 months, 4-6 months and 8-10 months) were used in this study. C57BL/6J mice were single-house mice, housed in a 12:12-h light-dark cycle and fed Purina rodent chow ad libitum. All procedures were performed in compliance with the IACUC of Southern Illinois University School of Medicine.

Immunohistochemistry (IHC): IHC was used to assess for neuropathology. Animals were perfused with 4% paraformaldehyde. Brains were extracted and post-fixed in 30% sucrose, sectioned at a 35 μm thickness and batch processed. To determine differences in Aβ accumulation, Mouse anti-β-42 (1:3000) and Rabbit anti-tau (pTau) clone PHF13 Millipore 5-885 1:3000 was used.

2.2 Methods

2.2.1 Glucose Tolerance Test (GTT):

Food intake and body weight were monitored for 7 days prior to GTTs. Following a 12 hour fast, baseline glucose readings were taken in duplicate using a handheld glucometer (Precision Xtra). Mice then received an intraperitoneal injection of a 20% glucose solution (1.5g/kg body weight). Glucose readings were taken in duplicate at 15,30,45,60, and 120 min. Blood samples were also collected at baseline and 15 min to analyze plasma insulin levels and secretion. Plasma insulin levels were assessed using an Ultra Sensitive Rat Insulin ELISA kit (Christyl Chem). Mouse anti-phospho-tau (pTau) clone PHF13 Millipore 5-885 1:3000 was used.

2.2.2 Insulin Sensitivity Test:

Insulin Tolerance Test (ITT): Mice were fasted for 5 hours prior to ITTs. Baseline glucose readings were then taken in duplicate, after which the mice received an i.p. injection of insulin (0.25U/kg body weight). Further glucose readings were taken every 15 minutes for 1 hour.

2.2.3 Morris Water Maze:

Impairments in spatial learning and memory were seen in aged 3xTg mice. This suggests that alterations in glucose tolerance could be a driving force for the AD pathogenesis.

Summary and Conclusions

• 2-month old 3xTg mice show decline in glucose tolerance as they age; however, 3xTg mice are always more hyperglycemic.
• 3xTg mice exhibit a reduction in basal insulin levels and a reduced capacity to secrete insulin following a glucose bolus, suggesting that altered insulin production/signaling could be responsible for impaired glucose tolerance.
• 3xTg and control mice respond similarly to exogenous insulin using an insulin tolerance test at all ages examined.

• As shown in Figure 7, impairments in glucose tolerance precede the neuroanatomical changes as well as behavior in 3xTg mice.

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References


Figure 1: Glucose tolerance curve for 2 month old 3xTg mice and controls.

Figure 2: Glucose area under curve (AUC) for 2, 4-6 and 8-10 month old 3xTg mice and controls.

Figure 3: No differences were seen in insulin sensitivity for 3xTg mice compared to controls at all ages

Figure 4: Insulin sensitivity curves for 2 month old 3xTg mice and controls.

Figure 5: Immunohistochemical staining for Pan-Aβ, phosphorylated tau and Aβ42 for 2 month old and 14 month old 3xTg mice.

Figure 6: Morris Water Maze

Figure 7: Time courses for glucose tolerance, neuroanatomical changes, and behavior for 3xTg mice.

Figure 8: Average swim time in the Morris water maze for 3 month old and 15 month old 3xTg mice (n=6 ANOVA, p<0.05)