Regional glutamate alterations in 11-month-old Tg2576 mouse model of Alzheimer's disease detected by in vivo 1H magnetic resonance spectroscopy at 9.4 T

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Introduction

Alzheimer's disease (AD) is characterized by progressive loss of multiple cognitive functions including memory impairment [1]. Recent studies suggest for both cholinergic and glutamatergic involvement in the etiology of Alzheimer's disease [1]. Glutamate (glu), a major excitatory neurotransmitter in the brain, plays an important role in memory formation. Previous magnetic resonance spectroscopy (MRS) studies have reported a decrease in level of Glu in AD brain, but the numbers of these studies were limited since the conventional proton (^{1}H) MRS at 1.5 T cannot reliably detect the glutamate + glutamine (glx) resonance in vivo [2, 3, 4]. Transgenic mouse models of AD offer the possibility to observe correlation between memory impairment, glutamate level and plaque load in vivo by using magnetic resonance imaging and spectroscopy at ultrahigh magnetic field strengths (e.g. 9.4T). Tg2576 mouse is one of the most commonly used models of AD and it shows age-dependent increase of amyloid plaques beginning at 9–11 months [5, 6]. This model has shown impairment in memory and learning already before 11 months of age. [6]. Till now in vivo analysis of glu and glx levels in different regions of the 11-month-old Tg2576 mouse brain has not been attempted by MRS. On the basis of the previous reports, we hypothesized that impairment in memory of 11-month-old Tg2576 mice might be associated with regional alterations in glu and glx levels. In this study, we applied in vivo ^{1}H MRS to monitor the regional changes in glu/tCr and glx/tCr in 11-month-old Tg2576 brain. Our results clearly show that there is a significant decline, both in glu/tCr and glx/tCr in the cortex region of the brain of Tg2576 mice as compared to age-matched non-transgenic litter mates. The early decrease in glutamate level in the cortex regions might be associated with cognitive decline observed in this mouse model of AD.

Methods

Ten Tg2576 mice [6] and 9 none-transgenic littermates **(**WT) were used in this study. The transgene is expressed in C57B6/SJL F1 mice, backcrossed to C57B6 breeders. The N2 generation mice of both genders were studied at the age of 11 months. All MR measurements were performed at 9.4 T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1 T/m gradients. A 20-mm birdcage radio-frequency (RF) coil was used for µMRI and MRS study. Before MR imaging, the mice were initially anesthetized with 2 % isoflurane (Forene, Abott, UK), in air (0.3 L/min) and oxygen (0.3 L/min) and maintained between 1- 1.5 % isoflurane during all procedure. While inside the probe, the respiration rate of the mouse was constantly monitored (Bio-SAM monitoring system. The MR images were acquired using the rapid acquisition with the relaxation enhancement sequence as described previously [7]. *In vivo* spectroscopic data were acquired from the same animals at age 11 from, cortex (ctx), cortex + hippocampus (ctx hipp), hippocampus (hipp), and thalamus (thal) regions using the point resolved spectroscopy sequence with TE = 15 ms, TR= 3500 ms, number of averages (NA) = 512. The voxel sizes were 8 µl for all regions except for ctx (12 µl). Metabolite concentrations were quantified using the LC model [8] and the unsuppressed water signal was used as an internal reference. Metabolites quantified with Cramer-Rao lower bounds (CRLB, estimated error quantification) ≤ 21% were classified as detected. Data from the WT and Tg2576 mice were compared using two-tailed student's T-test. Statistical significance was assigned for P values < 0.05.

Results

In vivo analysis of glutamate levels in WT and Tg2576 mice in different brain regions (ctx, ctx hipp, hipp, and thal) is shown in Fig. 1. A significant decrease in glu/tCr and glx/tCr was clearly observed in ctx region in transgenic mice as compared to WT mice (Fig. 1 a). A trend of decrease in the level of glu/tCr and glx levels was also evident for hipp, hipp ctx and thal regions (Fig. 1 a, b, c). It is known that prefrontal cortex plays an important role in short-term memory. We speculate that memory impairment observed in the Tg2576 mouse model of AD can be correlated with decrease in glu and glx/tCr in the cortex region. Although the plaque deposition begin to appear at the age between 9-11 months [5,6], the severity of plaque deposition is evident only after 14 months, in the cortex region of the Tg2576 mouse. Thus, a decrease in glutamate in the Tg2576 mouse brain at 11 month of age correlate better with decline in cognitive functions rather than severity of amyloid plaque deposition. Further work is needed to investigate possible correlations between early change in glu, glx levels, plaque load and memory function.

References:

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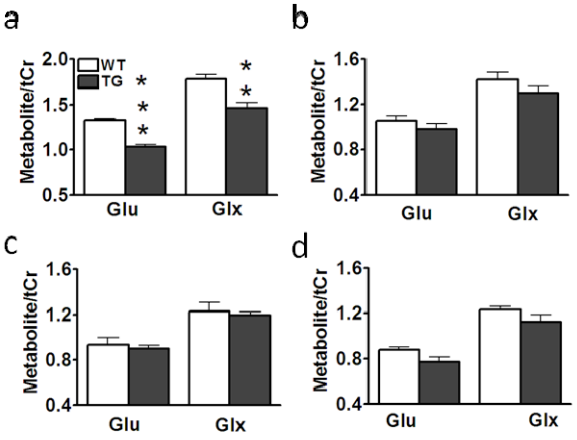

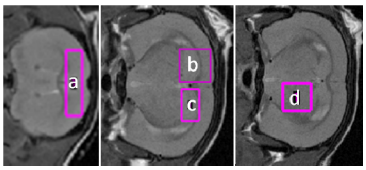

Fig. 2: The ^{1}H MRS voxel, outlined in pink, is positioned within (a) ctx, (b) ctx + hipp , (c) hipp, and (d) thal regions of the mouse brain. *Ctx*, cortex, *ctx+ hipp*, cortex + hippocampus, *hipp*, hippocampus, and *thal,* thalamus.

Fig. 1: Changes in neurochemical profile monitored by in vivo MRS in (a) ctx, (b) ctx + hip, (c) hipp, and, (d) thal regions in WT (open bars) and TG (gray bars) mice measured at age of 11 month. Error bars shown are standard deviation of mean. Statistically significant differences between TG and WT are shown with; * P<0.05, **P<0.01, *** P<0.001. *Ctx*, cortex, *ctx+ hipp*, cortex + hippocampus, *hipp*, hippocampus, and *thal,* thalamus, TG, Tg2576, WT, non-transgenic mice. The number of animals per group is as follow: (a) TG = 8, WT = 4; (b), (c), and (d) TG = 10, WT = 8.

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