Increased Glial Energy Metabolism during Normal Brain Aging assessed by Dynamic ^{13}C NMR Spectroscopy

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Introduction

Alterations in energy metabolism with aging have been implicated as a key factor in the etiology of many neurodegenerative diseases. However other than regional declines in CMRglc and $CMRO_2$ the metabolic changes in the aging brain have not been measured directly. In this study, ^{13}C NMR spectroscopy was combined with infusions of [1-^{13}C]glucose and [2-^{13}C]acetate to characterize quantitatively neuronal and astrocytic TCA cycle (V_{TCAn},V_{TCAa}) as well as the glutamate-glutamine cycle (V_{NT}) rates in healthy elderly and young volunteers.

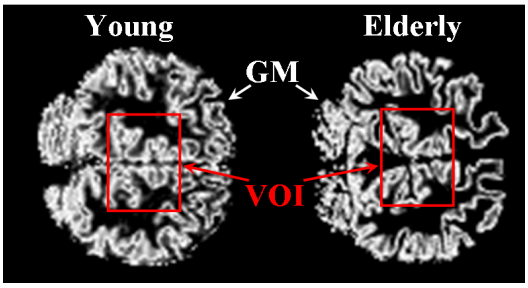

Fig. 1. Typical spectroscopic volume of interest (in red, ~100 mL) and extracted gray matter (GM) from a healthy young (left) and elderly volunteers (right).

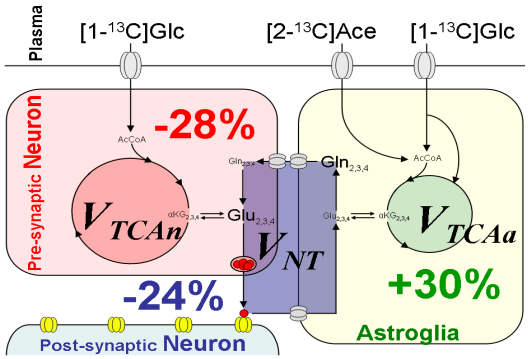

Fig. 2. Neuronal-astrocytic metabolic model and fluxes alterations with normal aging: V_{TCAn} (red), V_{TCAa} (green) and V_{NT} (blue).

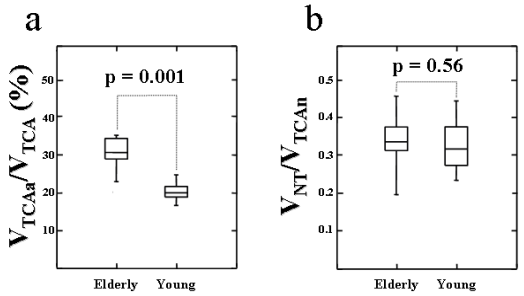

Fig. 3. A: V_{TCAa}/V_{TCA} and B: V_{NT}/V_{TCAn} ratios box-plotted for Young (n=8) and Elderly (n=7). Statistical Test: Non-parametric Kruskal-Wallis One-Way Analysis of Variance.

Materials and Methods

MRS acquisition. [1-^{13}C]glucose and [2-^{13}C]acetate infusion experiments were conducted on fifteen healthy volunteers, eight young (three females and five males; aged 26±7 years, BMI 23±4 kg/m^2; mean ± SD) and seven elderly (two females and five males; aged 76±8 years, BMI 24±3 kg/m^2; mean ± SD). This led to the composition of 30 experiments organized as 15 couples of ^{13}C NMR studies: 8 "Young" and 7 "Elderly". Subjects lay supine in a 4.0 T whole-body magnet (Bruker Instruments, Billerica, MA) with the head lying on top of one 8.5-cm-diameter ^{13}C circular coil and two ^{1}H quadrature coils for ^{1}H acquisition and decoupling. After tuning, acquisition of scout images, shimming with the FASTERMAP procedure [1], and calibration of the decoupling power, ^{13}C MRS spectra were acquired with polarization transfer [2] from a 50x40x45 mm^3 volume located in the occipital-parietal lobe before and during infusions of [1-^{13}C]glucose or [2-^{13}C]acetate (TR=2500ms, 128 transients). Infusion protocols for the [1-^{13}C]glucose and [2-^{13}C]acetate are described in detail elsewhere [2,3]. To determine the pools sizes of NAA, glutamate and glutamine for the "Elderly", short-TE ^{1}H spectra were acquired from the old volunteers using a LASER-POCE sequence (16 transients, TR=3.5s, TE=42ms) [4,5].

MRI segmentation. The tissue composition of each volume of interest (VOI) was determined from T_1-based image segmentation maps according to a previously described protocol [6,7] (See Fig.1).

MRS spectral analysis. Prior to processing, the ^{13}C scans were added in running averages of 15 min. All spectra were analyzed using LCModel 6.1 [8] (Stephen Provencher Inc., Oakville, Ontario, Canada) with simulated basis sets and modified input parameters for the ^{13}C spectra as described by Henry et al. [9]. Concentrations for the different isotopomers of glutamate and glutamine were obtained relatively to the signal of natural abundance NAA assuming a concentration of 11µmol.g^{-1} for the "Young" while the measured concentrations of NAA, glutamate and glutamine were considered for the "Elderly".

Metabolic modeling analysis. Combined datasets of ^{13}C labeling time-courses for Glu C4, C3 and C2, and Gln C4, C3 and C2 from [1-^{13}C]glucose and [2-^{13}C]acetate infusion experiments were fitted simultaneously according to a neuronal-astrocytic metabolic model [3,10,11]. Metabolic modeling was performed using Matlab 7.0 (The MathWorks Inc., Natick, MA) and CWave [12] with time-courses for plasma acetate, lactate and glucose concentrations and ^{13}C fractional enrichments as inputs functions for each experiment. Values for the rates of the glutamate/glutamine cycle (V_{NT}) along with the rates of neuronal and glial TCA cycles (V_{TCAn} and V_{TCAa}) were adjusted using a simulated annealing algorithm to find the best fit to each data set.

Results and Discussion

In spite of the overall brain atrophy with age, the proportion of gray and white matter in our volumes of interest were similar in both groups (Young: %GM=47±1% vs. Elderly: %GM=49±2%, mean±SD). As shown on figure 2, major metabolic changes were measured: 30% increase of V_{TCAa} (p=0.002), a 28% decrease of V_{TCAn} (p=0.013) and a 24% decrease of V_{NT} (p=0.03) (mean, non-parametric Kruskal-Wallis analysis). The large increase of astroglial contribution to total brain oxidative energy synthesis (Fig 3a: V_{TCAa}/V_{TCA} from 20±1 to 31±2%) is due to both an increase in V_{TCAa} and a decrease in V_{TCAn}. It may reflect increased astrocytic volume and metabolism in elderly probably linked to age-related reactive astrocytosis or microgliosis [13]. On the other hand, the correlated decrease of the glutamatergic neurotransmission and neuronal TCA cycle rates (Fig 3b: no significant change of V_{NT}/V_{TCAn} ratio, 0.32±0.03 vs. 0.34±0.03) might reflect mitochondrial dysfunction [14] as well as alterations of neuronal spines and synapses morphology and density [15].

Conclusion

In this study, we used MRI, ^{1}H and ^{13}C NMR spectroscopy to characterize quantitatively the evolution of brain energy synthesis capacity and neuronal-astrocytic relationship in the aging brain. Our data support the theory that alterations in oxidative energy production in both neurons and glia form a major characteristic of aging even in very healthy elderly subjects. The mechanisms leading to these changes remain to be identified but are consistent with reports from neuropathological studies and animal models of declines in mitochondrial density/function with aging.

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