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High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging

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ABSTRACT

Altered brain metabolism is likely to be an important contributor to normal cognitive decline and brain pathology in elderly individuals. To characterize the metabolic changes associated with normal brain aging, we used high-field proton magnetic resonance spectroscopy in vivo to quantify 20 neurochemicals in the hippocampus and sensorimotor cortex of young adult and aged rats. We found significant differences in the neurochemical profile of the aged brain when compared with younger adults, including lower aspartate, ascorbate, glutamate, and macromolecules, and higher glucose, myo-inositol, N-acetylaspartylglutamate, total choline, and glutamine. These neurochemical biomarkers point to specific cellular mechanisms that are altered in brain aging, such as bioenergetics, oxidative stress, inflammation, cell membrane turnover, and endogenous neuroprotection. Proton magnetic resonance spectroscopy may be a valuable translational approach for studying mechanisms of brain aging and pathology, and for investigating treatments to preserve or enhance cognitive function in aging.

1. Introduction

Understanding the neurobiological changes that occur with normal brain aging may aid in the prevention, diagnosis, and treatment of age-related cognitive decline and disease. In vivo proton magnetic resonance spectroscopy (^{1}H-MRS) allows noninvasive quantification of neurochemicals related to specific cellular mechanisms. These neurochemicals can serve as imaging biomarkers of cellular and molecular changes, both in the context of normal brain development and aging, and under pathologic conditions (Harris et al., 2012; Lee et al., 2012; Tkac et al., 2003). For example, N-acetylaspartate (NAA) is synthesized in neuronal mitochondria in an energy-dependent manner, so a decrease in NAA may indicate neuronal loss or metabolic dysfunction. Because the total choline (tCho) signal measured with ^{1}H-MRS is comprised largely of membrane phospholipids, increased tCho suggests cell membrane damage or increased turnover. Changes measured in glutamate (Glu) and/or its metabolic precursor glutamine (Gln) are interpreted as a sign of altered excitatory neurotransmission.

In humans, ^{1}H-MRS studies have reported that advancing age is associated with several neurochemical changes in the brain, including lower concentrations of NAA particularly in the frontal lobe, higher tCho in frontal and parietal regions, and higher total creatine (tCr) in the parietal and occipital lobes (reviewed in Haga et al., 2009). In addition, lower striatal Glu and higher myo-inositol (Ins) in the subcortical white matter, posterior cingulate, and hippocampus have been described in elderly individuals (Gruber et al., 2008; Kaiser et al., 2005; Reyngoudt et al., 2012; Zahr et al., 2013). However, it is not feasible to confirm the links between these brain chemicals and specific cellular and molecular mechanisms in humans.

In animal models of aging, not only can the potential variability in brain neurochemistry from genetic and environmental factors be

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strictly controlled, but the underlying mechanisms associated with altered brain neurochemistry can be established through histologic and biochemical assays. However, in vivo ^{1}H-MRS studies of aged animal models have been limited, and at times contradictory. A study by Katz-Brull et al. (2002) reported increased tCho in the hippocampus of aged rats, whereas Driscoll et al. (2006) observed no changes in NAA, tCr, or tCho in the aged rat brain. In vitro MRS studies of brain tissue extracts from rodents have measured additional neurochemical biomarkers. For example, an age-related increase in Ins suggests change in the density or metabolism of astroglial cells (Macri et al., 2006; Paban et al., 2010; Zhang et al., 2009) and altered Glu, aspartate (Asp), and gamma-aminobutyric acid (GABA), suggest a shift in the balance of excitatory and inhibitory neurotransmission in the aging brain (Paban et al., 2010; Zhang et al., 2009). However, one caveat of ex vivo MRS studies is that neurochemicals might be altered by the tissue extraction and homogenization process. A comprehensive in vivo assessment of neurochemical biomarkers of brain aging would be useful to facilitate translation of findings to clinical applications.

Compared with human studies, additional ^{1}H-MRS biomarkers can be measured in laboratory animals using higher magnetic field strengths and advanced spectral fitting techniques. Routine quantification of at least 18 neurochemicals in the rodent brain is now feasible with in vivo ^{1}H-MRS (Harris et al., 2012; Lei et al., 2009; Mlynarik et al., 2006; Pfeuffer et al., 1999). Such an expanded neurochemical profile has the potential to provide insight into cellular mechanisms, such as oxidative stress, inhibitory neurotransmission, and mitochondrial bioenergetics that might relate to changes in brain function with age. Thus, in the present study we characterized the metabolic effects of normal brain aging with in vivo ^{1}H-MRS at 9.4 tesla (9.4 T). Using validated, noninvasive methods (Harris et al., 2012), we measured 20 neurochemicals in young and aged Fischer (F344) rats. Because impaired memory and motor functions are common aspects of neurologic decline in normal aging (Schuff et al., 1999; Seidler et al., 2010; West, 1993), we acquired MRS from the hippocampus and the sensorimotor cortex.

2. Methods

2.1. Animals

Male F344 rats 2 to 3 months old ("young adults", n = 30) and 20 to 22 months old ("aged", n = 20) were used in the study. Animals were housed in pairs on a 12 hour light-dark cycle with free access to rat chow and water. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center and are consistent with standards of animal care set forth in the guidelines of the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.2. In vivo magnetic resonance imaging and spectroscopy

All magnetic resonance assessments were performed on a 9.4 T horizontal magnetic resonance (MR) system (Varian; Palo Alto, CA, USA). During imaging, anesthesia was maintained with 1.5%–3% isoflurane delivered via nosecone to maintain a respiration rate of 40–80 cycles/min. Respiration was monitored with a pressure pad (SA Instruments; Stony Brook, NY, USA). Animals were placed on a heating pad in the scanning cradle, and body temperature monitored rectally was maintained at 37 °C via feedback control (Cole Parmer; Vernon Hills, IL, USA).

Coronal and sagittal localizer MR images were acquired using a gradient echo multi-slice sequence to position the animal's head in the magnet (repetition time [TR] = 100 ms, echo time [TE] = 2.8 ms, number of slices = 10, slice thickness = 1 mm). Next, coronal and sagittal T_2-weighted MR images were acquired using a rapid acquisition with relaxation enhancement sequence (TR = 4000 ms, TE = 18 ms, echo train length = 8; averages = 2; field of view = 2.56 × 2.56 cm^2; resolution = 256 × 256 pixels; number of slices = 20; slice thickness = 1 mm) to position the voxels for ^{1}H-MRS.

^{1}H-MRS was acquired from a 2.7 mm × 1.3 mm × 2.7 mm voxel containing sensorimotor cortex, and a 3.0 mm × 2.5 mm × 3.0 mm voxel containing primarily hippocampus. Voxel positioning was based on anatomic landmarks. All spectra were acquired from the right hemisphere.

We used a custom-made quadrature dual-coil transmitter-receiver surface coil (each coil was 18 mm in diameter; Tkac et al., 1999). ^{1}H-MRS was performed using a stimulated echo acquisition mode (STEAM) sequence with variable pulse power and optimized relaxation delays (VAPOR) water suppression (TE = 2 ms, TR = 4000 ms; Tkac et al., 1999). First and second order shims were adjusted using FASTMAP (Gruetter, 1993) to achieve water linewidths of <18 Hz. Data were acquired as a series of free induction decays (each of which averaged 16 transients), corrected for frequency drift, averaged and corrected for eddy current effects. In the hippocampal voxel we averaged 320 transients over a period of approximately 20 minutes. In the smaller cortical voxel, we averaged 640 transients over approximately 40 minutes. We also acquired unsuppressed water scans (16 transients) from each voxel to use for concentration calculations described in the following.

2.3. Spectral fitting and quantification

Spectra were analyzed with LCModel (Provencher, 1993). LCModel uses a basis set of spectra to calculate the in vivo neurochemical concentrations, and the unsuppressed water signal from the prescribed voxel as a reference for each scan to correct for small variations in coil sensitivity as described previously (Pfeuffer et al., 1999). For specific neurochemicals, our basis set was acquired from in vitro samples of pure chemicals. The macromolecule basis set was measured empirically. Peak assignments for individual metabolites in the neurochemical profile were based on previous reports (Pfeuffer et al., 1999; Tkac et al., 2003). It has been confirmed that measurements of brain neurochemicals using this ^{1}H-MRS protocol are in good agreement with measurements from traditional invasive methods (Pfeuffer et al., 1999; Tkac et al., 2003). The following 20 neurochemicals were quantified: Ala, alanine; Asc, ascorbate; Asp, aspartate; Cr, creatine; GABA, gamma-aminobutyric acid; Glc, glucose; Gln, glutamine; Glu, glutamate; GPC, glycerophosphocholine; GSH, glutathione; Ins, myo-inositol; Lac, lactate; MM, macromolecules; NAA, N-acetylaspartate; NAAG, N-acetylaspartyl glutamate; PCho, phosphocholine; PCr, phosphocreatine; PE, phosphoethanolamine; Ser, serine; and Tau, taurine. Because certain neurochemicals that overlap at lower field strengths are often reported together, particularly in human studies, we report the following sums: tCr = Cr + PCr, tCho = GPC + PCho, returned by LCModel. We also calculated concentration ratios for certain pairs of metabolically-linked neurochemicals (i.e., Glu to Gln, and PCr to Cr).

2.4. Statistical analysis

Analysis of neurochemical concentrations was based on a weighted averages method. The LCModel software provides neurochemical concentration estimates and reliability measures expressed as Cramèr-Rao lower bounds (CRLB). Let x_i denote the measured concentration of a neurochemical X and r_i the corresponding reliability measure (CRLB), $i = 1, 2, \ldots, N$. Then the weight is computed as: