Regional and developmental variation in vitamin C concentration in the rat brain quantified in vivo using short echo-time ^{1}H MRS

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Introduction

The importance of vitamin C (ascorbate, Asc) in protecting the brain from oxidative injury is substantiated by: a high overall brain Asc concentration throughout life span, elevated levels present when anoxia is likely to occur (e.g. at birth, prior to arousal from hibernation, and in diving species) and intracellular concentrations proportional to the rates of oxidative metabolism in neurons versus glia (1). Several studies using ex vivo techniques have reported regional variation in rat brain Asc concentration (1-3) as well as region specific changes with postnatal development (1). The goal of this project was to determine whether region specific variation in Asc concentration could be quantified non-invasively during rat brain development using short echo time (TE) ^{1}H NMR spectroscopy.

Methods

Ultra-short TE STEAM spectra, previously acquired from the hippocampus, striatum, and cortex of developing rat pups at postnatal day 7 (P7) through P28 (spontaneously breathing anesthesia) (4) were reprocessed using LCModel (5) with an Asc spectrum added to the basis set. ANOVA was used to identify significant differences in Asc concentrations.

Results

Asc was reliably quantified from all 110 spectra (Cramer-Rao lower bounds 4 - 13%) even though the dominant Asc signal (at 3.73 ppm) overlapped strongly with resonances of other metabolites (Fig. 1). Asc was quantified among the five most concentrated neurochemicals (along with creatine, glucose, phosphorylethanolamine, and taurine) in the brains of P7 pups. Both postnatal age and brain region had an effect on Asc concentration ($P<0.001$). Concentrations were highest in all three regions on P7 and were indistinguishable between the three areas at this age ($P=0.14$). Concentrations decreased between P7 and P28 in all three areas ($P<0.001$). However, the decreases in the three areas were not uniform, such that beyond P7, Asc concentration differed among the brain regions at all 4 postnatal ages studied ($P\leq0.004$). See fig. 2.

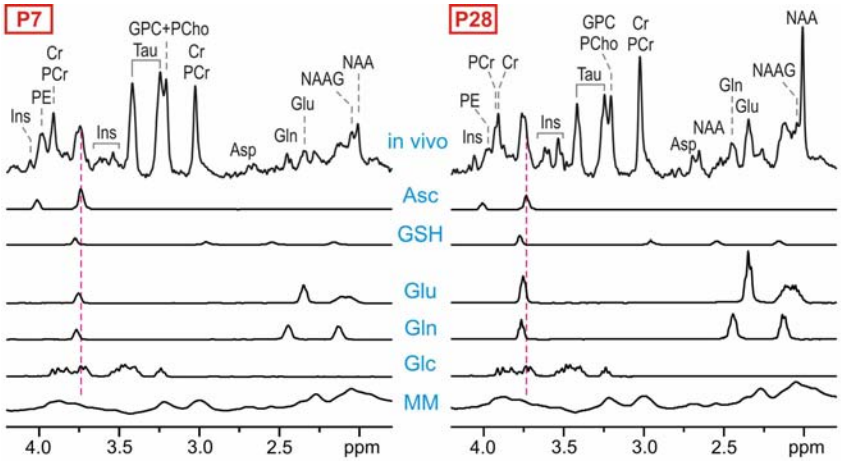

FIG. 1. LCModel analysis of in vivo NMR spectra (STEAM, 9.4 T, TE = 2 ms, TR = 5 sec, NT = 320) measured from striatum on P7 (18 µl) and P28 (24 µl) and spectra of the following metabolites strongly overlapping with Asc at 3.73 ppm: glutathione, glutamate, glutamine, glucose, and macromolecules.

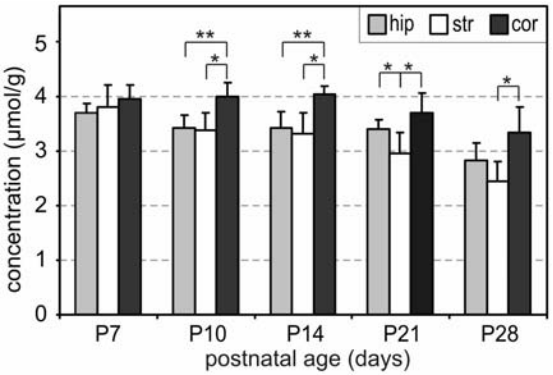

FIG. 2. Developmental and regional changes in Asc concentration (mean ± SD) in rat brain hippocampus (hip), striatum (str), and cortex (cor). A total of 110 spectra were analyzed with a minimum of 4 spectra per age and region group. To accommodate for growth, volume of interest (18-24 µl) dimensions increased with age (4). * $p < 0.01$, ** $p < 0.001$.

Discussion

The Asc concentrations quantified in this study agree per age and region of interest with previous in vitro (1-3) and in vivo (6) measurements and demonstrate feasibility to detect regional differences in brain Asc distribution. High cortical Asc concentrations correspond to high density of neurons, where Asc is predominantly localized (1). The pattern of similar concentrations at birth followed by regional divergence during development may explain region specific vulnerability to oxidative damage (7). This study demonstrates that the Asc concentration quantified from ^{1}H NMR spectra can be used for non invasive longitudinal evaluation of aberrant antioxidant capacity and efficacy of corrective procedures.

References (1) M. E. Rice et al, 2002, *Comp Biochem Physiol C* **133**:515 (2) R. Rajalakshmi et al, 1971, *Ind J Biochem Biophys* **8**:295 (3) K. Milby et al, 1982, *Neurosci Lett* **28**:15 (4) I. Tkac et al, 2003, *Magn Reson Med* **50**:24 (5) S. W. Provencher, 2001, *NMR Biomed* **14**:260 (6) M. Terpstra et al, 2006, *Magn Reson Med* **55**:979 (7) J. Towfighi et al, 1997, *Brain Res Dev Brain Res* **100**:149.

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