Neurochemical profile of the hibernating ground squirrel measured by ^{1}H NMR spectroscopy

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Introduction

Hibernation is a metabolic state in which the heart rate falls to a few beats/min and body glucose oxidation is reduced to a few percent of that in active animals, while the body temperature is maintained at ~5°C (1). Understanding how the mammalian brain adapts to a vast reduction in fuel consumption during hibernation may yield valuable insights into mechanisms to enhance neuroprotection after hypoxic-ischemia injury in the brain. The goal of our study was to measure the neurochemical profile in the active and hibernating 13-lined ground squirrel (*Spermophilus tridecemlineatus*) using ^{1}H NMR spectroscopy.

Methods

Squirrels were studied prior, during and after hibernation (n=7 for each group, of which 4 animals were studied longitudinally). During NMR measurements, all animals were anesthetized with 2% isoflurane in the circulating air. Body temperature was maintained at ~7^{0}C for the hibernators and ~37^{0}C otherwise. *In vivo* ^{1}H NMR spectra were obtained at 9.4T from an 18 µl volume in cortex using STEAM (TE=2ms, TM=20ms, TR=5 s) (2). Shimming of 1st and 2nd order shim coils was performed using FASTMAP. The basis sets for LCModel consisted of 20 metabolite spectra measured *in vitro* at 37°C (3). Because of the temperature dependence of chemical shifts and coupling patterns of typical brain metabolites (e.g. NAA, myo-Ins, PCr) an additional basis set for LCModel was measured at 7°C. Concentrations of 20 metabolites ("neurochemical profile") were obtained using quantitative LCModel analysis including an experimentally measured macromolecule basis spectrum. Tissue water was used as an internal concentration reference.

Results and Discussion

During hibernation, highly resolved *in vivo* ^{1}H NMR spectra were measured (Fig. 1) despite the low temperature and significantly increased linewidths. Additional challenges were presented due to the reduced relaxation times and the changes in chemical shifts with temperature (such as the NH of NAA at 7.8-8ppm in Fig. 1), requiring the measurement of a new basis set for LCModel at low temperature. Note that the water resonance was shifted downfield to 5.0 ppm as expected (Fig. 1). Applying the standard basis set to the spectra acquired during hibernation clearly produced non-zero residuals (Fig. 2d) that were removed once the appropriate basis set was used (Fig. 2c). Among the notable concentration changes include a visually apparent increase in PCr/Cr ratio from 0.94±0.26 to 2.16±0.50 (mean ± SD, $p<0.0001$) and an increase in GABA from 0.91±0.58 µmol/g to 2.13±0.36 µmol/g ($p<0.001$) during hibernation. These changes were reversed after terminal arousal from hibernation in spring.

In conclusion, hibernation was associated with significant changes in energy status and synaptic inhibition (GABA), whereas the putative neuronal marker N-acetyl-aspartate did not change substantially. The present results demonstrate the feasibility of obtaining quantitative results from hibernating brain. This study also underlines the importance of incorporating accurate prior knowledge, which can be gained from phantom studies, for the analysis of *in vivo* data using LCModel.

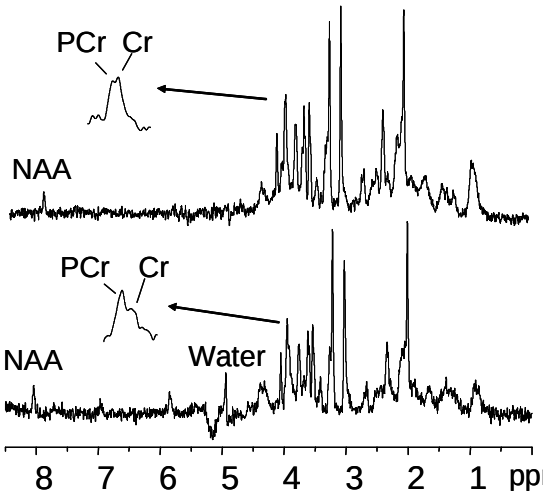

Fig.1. *In vivo* ^{1}H NMR spectra obtained from the brain of active (anesthetized) squirrel at 37°C (top) and hibernating squirrel at 7°C (bottom). Acquisition parameters: cortex 18 µl, 160 scans, TR 5s. Insets show changes in PCr and Cr resonances. The separation between Cr and PCr peaks around 3.9ppm was 7 Hz at 37°C and 10Hz at 7°C based on *in vitro* measurements.

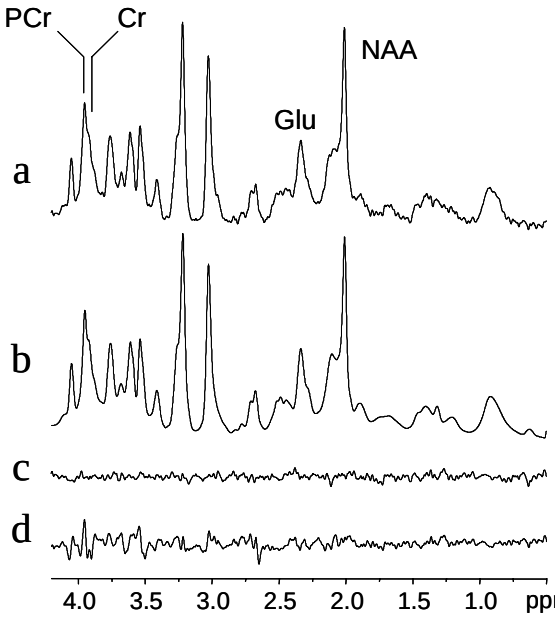

Fig.2. LCModel fit of an *in vivo* spectrum from hibernating squirrel brain at 7°C. (a) *in vivo* spectrum (b) LCModel best fit with the 7°C basis set (c) residuals when using the basis set measured at 7°C (d) residuals when using the basis set measured at 37°C.

References

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