Metabolic Changes in Rat Frontal Cortex after Injection of Pentylenetetrazole Measured by Proton MR Spectroscopy at 9.4T

S. Yang[1], D. Vaupel[1], H. Lu[1], T. J. Ross[1], W. Rea[1], S. M. Demny[1], E. A. Stein[1], and Y. Yang[1]

[1]Neuroimaging Research Branch, National Institute on Drug Abuse, NIH, Baltimore, MD, United States

Introduction

Pentylenetetrazole (PTZ) is a chemical convulsant frequently used in animal models to study seizures [1]. Microdialysis has been commonly employed to measure extracellular concentrations of metabolites and neurotransmitters after injection of PTZ, and a significant increase in glutamate (Glu) level has been demonstrated [2]. In this study, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to monitor potential changes of Glu, glutamine (Gln), and γ-aminobutyric acid (GABA) concentrations and their corresponding time courses during PTZ-induced seizures. After administration of PTZ, the neurotransmitter concentrations in the frontal cortex of adult rat brain were measured using localized ^{1}H MRS spectroscopy at 9.4 T.

Materials and Methods

Male adult Sprague-Dawley rat (265-370 g, n = 9) were anaesthetized using 2% isoflurane mixed with a 1:1 O_2/air gas mixture and the rats were orally intubated. A femoral arterial catheter was implanted for monitoring arterial blood pressure, blood gasses (pCO_2, pO_2), pH, and arterial blood pressure. A femoral vein was implanted with a double lumen catheter to administer the anesthetics. After surgery, rats were loaded into the animal holder on 2% isoflurane and then switched to and maintained on a constant i.v. infusion of Propoflo (40mg/kg/hour). Pancuronium Bromide (loading dose = 2mg/kg; maintenance dose = 2mg/kg/hour, i.v.) was administered for muscle relaxation. Eight rats were intraperitoneally injected with pentylenetetrazole (PTZ) (70mg/kg in 0.5 ml saline; 20 sec infusion) followed by a saline flush of the same volume. One rat died around 1 hour after injection of PTZ, so the data of this rat were excluded from the data analysis. One rat was injected with saline as a control.

All the ^{1}H MRS experiments were performed on a Bruker Biospin 9.4T scanner (Bruker, Karlsruhe, Germany). High-resolution T_2-weighted anatomical images were acquired by using a rapid acquisition with relaxation enhancement (RARE) sequence, TR/TE = 2000/50 ms, RARE factor =8. The anterior commissure was used as a reference point. A short-TE STEAM sequence was used to obtain the spectral data from a voxel of 3×3×3 mm^3 encompassing the cingulate and prelimbic cortex of rat brain. The sequence parameters were TR/TE/TM = 3000/9.1 (minimum)/20 ms, bandwidth = 10 kHz, sampling points = 4096, repetition = 500, a total time = around 25 min. Each FID was individually stored and the spectral raw data were corrected for eddy current (by turning on the eddy-current correction) and frequency shift (by turning on the retro frequency lock with the navigator scan). Spectral quantification was carried out in the LCModel analysis software [3] using unsuppressed water signal (from a separate scan) for scaling. An unsuppressed water scan (4 repetitions) was obtained ahead of each water-suppressed MRS scan of 25 min. The basis set consisted of the model spectra of alanine (Ala), aspartate (Asp), creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), Gln, Glu, glycerophosphocholine (GPC), phosphocholine (PCh), *myo*-Inositol (Ins), lactate (Lac), *N*-acetylaspartate (NAA), and *N*-Acetylaspartylglutamate (NAAG). A separate sub-set of macromolecules and lipid were also included in the basis set. Only the estimated concentrations with a Cramer-Rao Lower Bound (CRLB) less than 20% were included into the final data analysis. The time series of metabolite concentrations (3 time points before the injection and 4 time points after, 25 minutes for each time point) were normalized to the baseline level (an average of 3 time points of pre-injection data) and analyzed with a linear mixed model using SPSS v15. The model included one factor (pre vs. post injection) and a diagonal covariance structure for the repeated measurements over time.

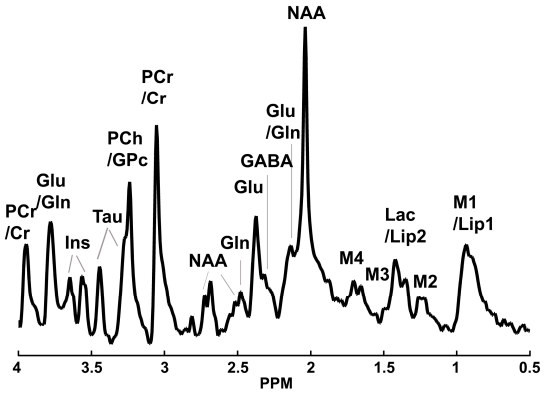

Fig. 1. A localized *in vivo* STEAM spectrum from a voxel encompassing cingulate and prelimbic cortex on a rat brain.

Results

Fig. 1 shows a localized *in vivo* STEAM spectrum from the specified voxel on a rat brain. The spectrum demonstrates good spectral resolution due to high spectral dispersion at 9.4T. The linewidth of unsuppressed water peak (obtained between each 25-min scan) remained constant between pre- and post-injection scans, suggesting little movement of rat due to the injection of PTZ. The following metabolites had the CRLB of less than 20%, GABA, Gln, Glu, Ins, NAA, GPC+PCh, NAA+NAAG, Cr+PCr, and Glu+Gln (Glx), with generally 3% for NAA, NAA+NAAG, and Cr+PCr, 3%-5% for Glu, GPC+PCh, and Glu+Gln, and 6%-9% for Ins, and 9%-14% for Gln and GABA.

Both GABA and Gln exhibited a post-injection increase in concentration [$F(1, 35.583)=6.130$, $p<0.018$ and $F(1,15.274) = 6.648$, $p<0.021$ respectively]. The averaged time series (1-3: baseline and 4-7: after injection) of GABA and Gln concentrations, normalized to the baseline level (obtained through averaging of three pre-injection time points individually on each rat), is shown in Fig. 2. Post hoc analysis indicated non-significant trends for time points 5 and 6 (25 and 50 minutes post injection) differing from baseline for Gln ($p<0.09$ and 0.07 respectively) and for time point 5 (25 min post injection) differing from baseline for GABA ($p<0.07$). No significant changes were found on other metabolite concentrations between pre- and post-injection of PTZ. For simplicity, the time series of these metabolite concentrations were ignored in Fig. 2.

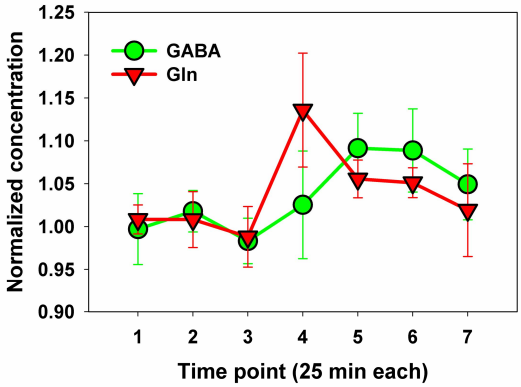

Fig. 2. The averaged time series (1-3: baseline and 4-7: after injection) of GABA and Gln concentrations, normalized to the baseline level that was obtained through averaging of three pre-injection time points individually on each rat.

Discussion

Microdialysis studies have shown increased Glu levels after administration of PTZ. However, microdialysis measures extracellular Glu concentrations, whereas MRS measures the entire Glu in the voxel. Since the Glu pool in the synaptic gap contains a very small fraction (~1%) of the total Glu, the increased extracellular Glu may not be detectable in the MRS. The increase of Gln may be attributed to the increase of Glu-Gln cycling rate after PTZ injection and Gln molecules are primarily located in the intracellular space. As shown in Fig. 2, the GABA level also increased after injection of PTZ but had a shifted peak time from that of Gln. As seizures are not constant but have ictal and interictal periods, the time-dependent changes of excitatory and inhibitory neurotransmitter levels may reflect this cycling. Further electrophysiological studies are needed to confirm the seizure patterns.

References

[1] Eloqayli H et al., J Neurochemistry 2003; 85:1200-1207. [2] Li Z et al., Neuroscience Letters 2000; 282:117-119. [3] Provencher SW, MRM 1993; 30:672-679.