Glutamate and Glutamine Changes Induced by Ethanol Treatment in the Rat Brain Detectable with CT-PRESS at 3T

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INTRODUCTION A variety of psychiatric disorders are associated with brain elevations in the combined resonances of glutamate (Glu)+glutamine (Gln)+glutathione (GSH)(i.e., Glx). For example, in rats exposed to vaporized ethanol (EtOH) in escalating doses, MRS reveals an increase in Glx in the basal ganglia [1]. However, Glx measurements blur the complex relationship between Glu and Gln. Subtle changes in Glu and Gln may have important implications to brain functioning. The current analysis was conducted to determine whether the signal from Gln could be quantified separately from that of Glu. The hypothesis tested was that the increase in Glx previously observed in response to EtOH is due to an increase in the levels of Gln, but not Glu.

MATERIALS AND METHODS The study group comprised 10 sibling pairs of healthy male, wild-type Wistar rats (~293g at the time of reception). After a baseline scanning session (MRS1), one rat from each sibling pair was exposed to a mixture of EtOH and air, and the other to air using a rodent alcohol inhalation system (La Jolla Alcohol Research Inc.). Animals received intermittent exposure to vaporized EtOH (14h at night) for 24 weeks. MRS was performed at week 16 (MRS2) when blood EtOH concentrations (BECs) averaged ~293mg/100ml and week 24 (MRS3) when BECs averaged ~445mg/100ml. Two EtOH rats died after the first 16 weeks of EtOH exposure. All MR data were acquired on a 3T GE Signa MR scanner equipped with a high-strength insert gradient coil (500mT/m, 1800mT/m/ms). A quadrature birdcage RF coil (i.d.=44mm) was used for both RF excitation and signal reception. Scans were ~2h each: anesthesia was provided by 2-3.5% isoflurane in oxygen (~1.5L/min). Spectra were acquired with CT-PRESS from a 10x5x5 mm^3 voxel in the basal ganglia. The sequence was optimized for the detection of Glu with acquisition parameters: t_c=139ms, $\Delta t_1/2$=0.8ms, n_1=129, 2048 complex points at SW_2=5000Hz, TR=2s, 6 averages, 26:36min [3]. A second acquisition without water suppression ($\Delta t_1/2$=6.4ms, n_1=17, 2 averages, 1:16min) was performed to determine tissue water content used as a reference for metabolite quantification. The data were phase corrected using the residual water signal, apodized with a 5Hz Gaussian line broadening, zero-filled to 8192 points, subjected to FFT along t2, then normalized to voxel tissue water [3]. To separately quantify Glu and Gln, time-domain data for metabolites of interest were generated using GAMMA simulation with a full density matrix and CT-PRESS timing parameters. Taking into account each metabolite's T2 relaxation time and dephasing due to B0 inhomogeneity, time domain basis sets were constructed by summation of an individual metabolite's data set multiplied by its concentration weight. Concentrations of NAA, Cho, and Cre were estimated by fitting the basis and measured spectra at their chemical shift. Glu concentration was estimated by fitting its resolved C4 resonance at 2.35ppm. Gln C4 resonance was not resolved and overlapped with the coupled NAA resonance at 2.45ppm. However, since the coupled NAA resonance was determined in the basis set, Gln concentration was estimated by fitting at 2.45ppm. The estimated metabolite concentration data were subject to a 2 group-by-3 time point analyses of variance (ANOVA); results of interest were group effects and their interactions, especially as related to Glu and Gln. Follow-up between-group differences were conducted with t-tests (1-tailed for predicted directions).

RESULTS ANOVA indicated a group-by-time-by-metabolite interaction ($F(10,160)=3.7$, $p=.0002$). Between-group differences for all metabolites followed a similar pattern across the 3 time points as previously observed [1]. However, while the previous analysis suggested a trend towards lower levels, the new analysis revealed that both NAA ($t(16)=2.5$, $p=.01$) and Cre ($t(16)=3.3$, $p=.002$) in the EtOH group were indeed significantly lower than in the Con group at MRS3. At MRS3, after 24 weeks of vaporized EtOH exposure, the EtOH group had higher levels of Glu ($t(16)=2.62$, $p=.009$) than the Con group as previously noted [1]. The novel finding presented here is the observation of higher levels of Gln in the EtOH group compared with the Con group ($t(16)=3.61$, $p=.0012$) after 16 weeks of EtOH exposure (MRS2; Figure 1).

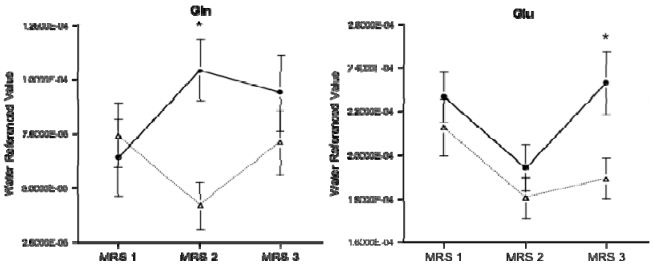

Figure 1. Mean±SEM of Glu and Gln quantified relative to tissue water for each of the 3 MRS acquisitions for control (open triangles) and EtOH-exposed (closed circles) rats. *p≤.05

DISCUSSION AND CONCLUSION These findings corroborate a recent in vitro NMR study in EtOH-exposed rats [4]. Analysis of Glu and Gln separately unveils changes indistinguishable using the combined marker Glx, although the significant contribution to Glx from the GSH singlet remains unaccounted for. While our previous analysis indicated a dose-dependent increase in Glx, the current results reveal an increase in Gln at MRS2 and an increase in Glu at MRS3. Increases in brain Gln are reported in patients with hepatic encephalopathy upon postmortem examination [5]. Evidence for mild liver damage in the EtOH group [1] and for elevations in blood ammonia with EtOH exposure [6] suggests an explanation for elevated Gln: liver failure impairs the major organ for ammonia elimination via the urea cycle, and the consequent increase in blood ammonia levels can lead to elevated brain ammonia. The mechanism of brain ammonia detoxification is the formation of Gln from Glu by the enzyme Gln synthetase [7]. With prolonged EtOH exposure, however, the levels of Gln synthetase may be compromised [8], leading instead to a build-up in the levels of Glu as observed after 24 weeks of EtOH exposure. Given the decline in NAA, the current results may imply that elevated Glu levels mediate brain EtOH toxicity [e.g., 9].

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