Evidence that Exposure to Escalating Doses of Vaporized Alcohol Causes Increase in the Concentration of Choline-Containing Compounds in the Basal Ganglia of the Rat

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Introduction

In a recent MRS study of rats exposed to ethanol vapor we found an increase in the signal from choline-containing compounds (Cho) in the basal ganglia with escalating alcohol exposure [1]. Data were acquired with constant time point-resolved spectroscopy (CT-PRESS) [2] and metabolite signals were evaluated in the diagonal spectrum because of its improved spectral resolution and high signal-to-noise ratio (SNR) for signals from J-coupled resonances. CT-PRESS data were acquired with an average echo time (TE) of 139 ms, which is in the range of echo times where transverse relaxation considerably affects the metabolite signal intensities. Therefore, the change in Cho signal could be explained by a change of the transverse relaxation constant (T_2) of Cho rather than change in Cho concentration. Because data were acquired at different TEs, T_2s for the high SNR signals from N-acetyl-aspartate (NAA), total creatine (tCr), and Cho could be estimated from the CT-PRESS data. Thus, this analysis sought to determine whether the previously identified group differences based on conventional CT-PRESS analysis were attributable to alcohol-exposure differences in concentration or, alternatively, T_2.

Materials and Methods

The study group comprised 10 sibling pairs of healthy male, wild-type Wistar rats weighing (293 ± 38) g at the time of reception. After the pre-alcohol baseline scanning session (MRS1), one rat from each sibling pair was exposed to a mixture of alcohol and oxygen, the other to oxygen, using a rodent alcohol inhalation system (La Jolla Alcohol Research Inc.). Concentrations of alcohol vapor were adjusted by varying the rate at which alcohol was pumped into the flask and ranged from 15 to 30 mg/l. Chambers administering intermittent alcohol vapor were connected to a timer that would turn the chambers on and off every day so that animals received alcohol for 14 h at night. Rats were exposed to vaporized alcohol for a total of 24 weeks; MRS was performed at week 16 (MRS2) and week 24 (MRS3). All MR data were acquired on a 3T GE Signa MR scanner equipped with a high-strength insert gradient coil (500 mT/m, 1800 mT/m/ms). A quadrature birdcage RF coil (∅ = 44 mm) was used for both RF excitation and signal reception. Rats were scanned in sessions of ~2h each while anesthesia was provided by 2-3.5% isoflurane in oxygen (~1.5 l/min).

Spectra were acquired with CT-PRESS from a $10(x)\times5(y)\times5(z)$ mm^3 voxel in the basal ganglia. The sequence was optimized for the detection of glutamate with acquisition parameters: t_c = 139 ms, $\Delta t_1/2$ =0.8 ms, n_1 = 129, 2048 complex points at SW_2 = 5000 Hz, TR = 2 s, 6 averages, 26:36 min [3]. A second acquisition without water suppression ($\Delta t_1/2$ = 6.4 ms, n_1 = 17, 2 averages, 1:16 min) was performed to determine tissue water content used as a reference for metabolite quantification. For each TE step, only the time domain data from each TE onward was used. The data were phase-corrected using the residual water signal, apodized with a 5 Hz Gaussian line broadening, and zero-filled to 8192 points. After FFT along t_2, the spectra were normalized to the amount of tissue water in the voxel as calculated from the data acquired without water suppression [3]. Signals from NAA, tCr, and Cho were evaluated at each TE by peak integration with an integration interval of ±9 Hz. Metabolite T_2s and signal amplitudes corrected for transverse relaxation were estimated by linear regression of the semi-logarithmic data. Only data TE > 49 ms were used in the fit to reduce contributions of macromolecule resonances. Simultaneously, data with TE > 221 ms were excluded due to low SNR.

Each set of metabolite data was subject to a series of 2 group-by-3 time point analyses of variance (ANOVA); results of interest were group-by-time interactions, especially related to Cho, which had been previously identified as the only major proton metabolite to show an interaction related to alcohol exposure. Two rats died after the first 16 weeks of alcohol exposure and one of the control animals was excluded from the analysis because it was not possible to get reliable T_2 estimates at MRS2.

Results

We tested the hypothesis that T_2 values of the metabolites would not show group or group-by-time effects, whereas Cho signal corrected for echo time would show this interaction. In support of our hypothesis, ANOVAs for T_2s did not yield any significant group-by-time interactions. By contrast, ANOVAs of metabolite concentrations revealed an interaction for Cho ($F(2,45)=4.46$, $p=.0171$) but not NAA ($p=.496$) or tCr ($p=.642$).

Discussion and Conclusion

The presented data provide further confirmation for our conclusion that the increase in Cho signal observed with increasing exposure to vaporized alcohol was due to an increase in metabolite concentration rather than a change in T_2. Another factor that could have affected these results was a contribution from taurine (Tau) to the Cho peak; however, the Tau peak at 3.4 ppm, which was not overlapping with Cho, did not show a group-by-time interaction in the conventional CT-PRESS analysis. A remaining possible contribution to the change in Cho signal arises from the T_1 of the Cho signal. Increased Cho could be explained by a shortening of T_1, because of the incomplete longitudinal relaxation at the chosen TR of 2 s. Nonetheless, the current analysis provides converging evidence that brain Cho is affected by high doses of alcohol. In other conditions affecting the brain, including multiple sclerosis, HIV infection, and normal aging, elevated Cho has been interpreted as indicative of demyelination, inflammation, or abnormally high glial density [4,5].

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References

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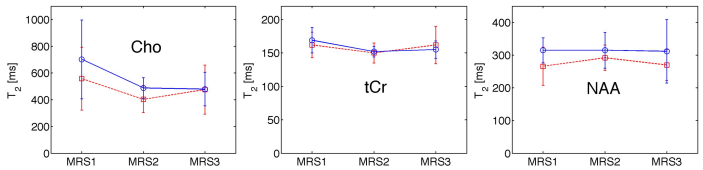

Fig. 1: Mean±STD of metabolite T_2s for each of the three MRS acquisitions for the alcohol-exposed (blue circles) and control (red squares) rats.

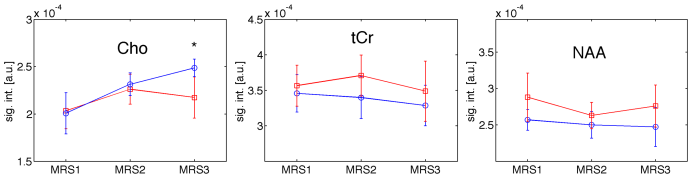

Fig. 2: Mean±STD of metabolite signal corrected for TE for each of the three MRS acquisitions for the alcohol-exposed (blue circles) and control (red squares) rats.