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Long term antipsychotic treatment does not alter metabolite concentrations in rat striatum: An in vivo magnetic resonance spectroscopy study

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ABSTRACT

Proton magnetic resonance spectroscopy (MRS) studies of schizophrenic patients generally reveal reduced levels of N-acetyl aspartate (NAA) when compared with healthy controls. Whether this reduction is due to the disease or to the drugs used for treatment remains an open question. Numerous human and animal studies have attempted to determine the effects of antipsychotics on NAA levels with mixed results. The majority of the animal studies were ex vivo, which may not accurately reflect the in vivo situation, and limitations of the human studies include previous or concomitant medications or other confounds. To overcome these limitations, we dosed 10 rats/group for six months via drinking water with 0.2 or 2 mg/kg/day haloperidol or 10 or 30 mg/kg/day clozapine. Control rats received unadulterated water. Proton MRS data were collected longitudinally over the six month period from a 64 μL voxel containing primarily the right striatum prior to and monthly during drug administration and used to estimate the concentrations of NAA, creatine, and choline. Ratios of NAA, choline, inositol and glutamate + glutamine to creatine were also calculated. Only the Cho/Cr ratio showed a significant time-by-treatment effect ($p = 0.0285$). These results are in agreement with previous studies of the striatum. However, regional and disease-specific effects remain unresolved.

1. Introduction

Proton magnetic resonance spectroscopy (1H-MRS) studies of patients with schizophrenia generally report reduced levels of the metabolites N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myoinositol (Ins), glutamate (Glu), and glutamine (Gln) in various brain regions (Brugger et al., 2011; Chang et al., 2007; Steen et al., 2005; Theberge et al., 2004; Theberge et al., 2007). These metabolites are easily detected and quantified in proton MRS spectra from human brain and reflect aspects of underlying metabolic processes. NAA is generally considered a marker of neuronal health, Cr energy metabolism, and Cho membrane synthesis or turnover (Ross and Sachdev, 2004). NAA levels tend to be decreased in the frontal and temporal lobes, thalamus, and cerebellum of schizophrenic patients (see, for example, the meta analyses by Steen et al., 2005 and Brugger et al., 2011). Reductions in Cr and Cho have been reported in prefrontal cortex and thalamus (Yoo et al., 2009). Olbrich et al. (2008) report elevated glutamate + glutamine (GLX) levels, while others report increased Glu/Gln ratios (Bustillo et al., 2010; Shirayama et al., 2010). However, Tayoshi et al. (2009) reported decreased Glu.

Schizophrenic patients usually are stabilized on antipsychotics when they participate in MRS studies. Therefore, changes in metabolite concentrations could result from the disease and/or the antipsychotics. Different medications target different receptor systems and might have different effects on brain metabolism. Studies of the effects of antipsychotics on MRS measures of metabolite levels are problematical because patients are often switched from one medication to another to optimize their treatments, have been on other antipsychotics for some time preceding a variable washout period, or are on concurrent medications to treat other symptoms. Other confounds include diet, socioeconomic status, and substance abuse. This variability in patient subjects makes it difficult to identify the effects of any specific drug.

Animal studies are useful in attempting to differentiate medication effects because there are no confounds associated with exposure to other medications, lifestyle, or the clinical need to adjust medications. The reported results from studies on the effects of antipsychotics in rat brain are often contradictory, which may be due to the methods employed, such as ex vivo techniques that may not accurately reflect in vivo MRS measurements. For example, lactate concentrations rise rapidly post-mortem, so the increased lactate reported by McLoughlin et al. (2009) for some drugs may be more related to the post-mortem interval than the medication. Drug doses were not standardized in these studies, several doses were above clinical standards, and none examined dose-level effects. Only two of the previous studies examined the effects of chronic treatment and none of the treatments examined multiple time points.

Here we report the results of a six-month in vivo MRS study of metabolite levels in the right striatum of normal rats given one of two

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doses of either clozapine or haloperidol. Time constraints limited us to one brain region, so we chose striatum for comparison with our previous results (Lindquist et al., 2000) and because Harte et al. (2005) reported an NAA increase only in the striatum.

2. Materials and methods

2.1. Animals

The Institutional Animal Care and Use Committee approved these experiments, which were performed according to National Institutes of Health guidelines. Four groups of 6-week-old male Sprague–Dawley rats (n = 10/group) were given haloperidol (0.2 mg/kg/day or 2 mg/kg/day) or clozapine (10 mg/kg/day or 30 mg/kg/day) via drinking water for 6 months. Control rats (n = 10) were given water. Haloperidol was obtained from Aldrich. Clozapine was obtained from the NIMH chemical synthesis and drug supply program. Stock drug solutions were made by dissolving each drug in dilute HCl and adjusting the pH to 5 using dilute NaOH. The stock solution was diluted as needed to make 500 ml drinking water at the desired concentration for each rat.

Animals were housed singly so the volume of water consumed per animal could be measured. Animals were placed on a 25% restricted diet to reduce growth and ensure the animals would fit in the radiofrequency coil used for the MR experiments for the duration of the experiments. Animals were weighed weekly and the drug concentration in the water adjusted to maintain the desired dose-by-weight. Water was changed twice weekly.

Due to limitations on scanning time, the animals were divided into 3 cohorts of 8, 25, and 17 animals containing approximately equal numbers of each treatment group.

2.2. MRS data acquisition

Animals were anesthetized with 5% isoflurane in air and positioned prone on a custom-built holder with their teeth fixed in a bite bar. The rat and holder were then positioned in the center of a 38 mm Litz coil (Doty Scientific, Inc., Columbia, SC). Animals were maintained with 1.5% isoflurane in air during scans. Respiration rate and air temperature were monitored using equipment from Small Animal Imaging, Inc (SAI, Inc., Stony Brook, NY). The air temperature was automatically maintained at 30 °C by a flow of warm air around the animal. Respiration was maintained between 30 and 60 breaths per minute by adjusting the amount of isoflurane.

Data were acquired from each rat prior to and monthly during antipsychotic dosing using a 7T Bruker BioSpec system (Bruker, Ettlingen, Germany). Axial and sagittal localizer images were acquired for use in positioning the voxel. Spectra were acquired from a 64 μL cubic voxel primarily in the right striatum (Fig. 1A). After shimming on the voxel, a double spin echo sequence was used to acquire spectra with (128 averages) and without (4 averages) water suppression at an echo time of 20 ms and a repetition time of 6000 ms. Additional unsuppressed water spectra were acquired at 12 different echo times to calculate the fraction of CSF in the voxel. Eddy currents and phase were corrected with Bruker post-processing routines. Total scan time was approximately 2 h.

2.3. Metabolite concentration analysis

Data were imported into LCModel (Provencher, 1993) to estimate concentrations of NAA, Cr, Cho, glutamate + glutamine (GLX) and Ins referenced to brain water (assumed to be 43.3 mM/g). Results were used if the Cramer–Rao lower bounds were less than 20% for all metabolites except Ins, where a cut-off of 30% was used. The increased cut-off was used because the coupling pattern of Ins becomes significantly more complex at 7T than at lower field strengths, making fitting of this metabolite more difficult. Relaxing the Cramer–Rao lower bounds ensured that there would be sufficient Ins estimates for statistical analysis. LCModel concentration estimates were corrected for the gains and number of averages used to acquire the suppressed and reference data. No corrections were made for relaxation times; results are reported in institutional units.

2.4. Statistics

R (www.r-project.org) was used for all statistical analyses. Weights and water consumption at study start were analyzed using one-way ANOVA. Changes with time or treatment for weight, water consumption, and metabolite levels were analyzed using linear mixed models with the lme package in R. Time, treatment, and cohort were factors. If the lme analysis indicated an effect of time or treatment, the estimable function was to determine the source of the effect. Reported p-values are not corrected for multiple comparisons.

3. Results

There were no significant differences in the initial weights, nor were there significant treatment, cohort, or cohort interactions. There were significant day ($p<0.0001$) and day-by-treatment effects ($p=0.0124$). Individual comparisons revealed that the rate of weight gain for the low-dose clozapine group was significantly greater than that of controls ($p=0.0020$).

There were no significant differences between treatment groups in water intake at the study start. There was a significant day-by-treatment effect ($p=0.0465$). Further analysis revealed that the rate of water intake for the low-dose clozapine group differed from controls ($p=0.045$).

Typical LCModel fits for one rat over the entire treatment period are shown in Fig. 1. Approximately 20% of the data were discarded due to instrument malfunctions or high Cramer–Rao lower bounds in the fitted data. Plots of the monthly concentration data are shown in Fig. 2. Linear mixed model analysis of the concentration data indicated a significant effect of time for all measured metabolites, a treatment effect only for GLX ($p=0.029$), and no time-by-treatment effects. Monthly concentration estimates for each of the treatment groups are given in Table 1.

Metabolite ratios to Cr were calculated from the retained LCModel fits; plots of these data are shown in Fig. 3. Linear mixed models analysis of the ratio data indicated no significant treatment effects and significant time effects for NAA/Cr ($p=0.007$) and GLX/Cr ($p=0.034$). Only Cho/Cr ($p=0.0285$) showed a significant time-by-treatment interaction.

4. Discussion

Multiple studies of the frontal lobe report no changes in metabolite levels that correlate with medication status (Bustillo et al., 2008, 2010; Pae et al., 2004; Szulc et al., 2005, 2007). Bustillo et al. (2002) reported reductions in frontal NAA levels in patients after a year of haloperidol or quetiapine treatment, but did not differentiate between the antipsychotics or between treatment effects and disease progression. Conversely, Choe et al. (1996) observed increases in NAA/Cr in 13/34 patients following treatment with any antipsychotic. Bertolino et al. (2001) reported increased NAA/Cr in the dorsolateral prefrontal cortex in chronically ill patients following treatment regardless of antipsychotic. In one of the few studies where medication changes did not occur during the study, the NAA/Cr ratio increased after 8 weeks of clozapine therapy (Ertugrul et al., 2009). However, the patients in this study were refractory to other medications, so differences due to disease severity or lingering effects from the previous medications cannot be excluded.