Evidence of Altered Membrane Phospholipid Metabolites in Children with Attention Deficit-Hyperactive Disorder (ADHD): An *In Vivo* ^{31}P Spectroscopy Study

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INTRODUCTION

The neurodevelopmental disorder, attention deficit-hyperactive disorder (ADHD), is one of the most prevalent childhood behavioral disorders, affecting approximately 7% of the U.S. population. ADHD is first diagnosed in children with symptoms of inattention, hyperactivity and impulsivity. The more consistent neuroimaging findings have implicated the basal ganglia (BG) and prefrontal (PF) regions, which are regions associated with the neural networks of attention; however the molecular/biochemical underpinning(s) causing ADHD has yet to be fully understood. The purpose of this study is to use *in vivo* phosphorus (^{31}P) spectroscopy [a noninvasive technique that can directly assess the metabolism of membrane phospholipids (MPL) and high-energy phosphates in multiple, localized brain regions] to assess possible molecular/biochemical alterations in children with ADHD compared to age- and gender-matched controls. We hypothesize MPL metabolite deficits in the PF and BG regions suggesting a lack of development or an underdevelopment of neuronal processes and synapses in ADHD.

SUBJECTS AND METHODS

Eleven children with DSM-IV ADHD (10 males and 1 female; mean age 9.7±1.5 years; age range 7.0 to 11.9 years; 7 with the combined type and 4 with the predominantly inattentive type; and all but two have a comorbid oppositional defiant disorder) and 12 healthy, normal control subjects (10 males and 2 females; mean age 9.7±1.9 years; age range 6.6 to 12.2 years) participated in this study. Eight ADHD children refrained from taking the stimulant medication at least 24-hours prior the scan, 2 ADHD children were on stimulant medication at time of scan, and one subject was stimulant-naïve. *None of the ADHD subjects have a first-degree relative diagnosed with a major psychiatric disorder.*

A doubly tuned transmit/receive volume head coil was used to acquire the multi-voxel ^{31}P spectroscopy data on a GE LX 1.5 T whole body MR imager. Based on sagittal scout images, the CSI slice was positioned parallel with the anterio commissure-posterior commisssure line to include the right and left PF and BG regions. A single slice selective excitation RF pulse followed by phase encoding pulses to spatially encode the two dimensions within the axial slice (termed FIDCSI on a GE system) was used to acquire the ^{31}P CSI data. The experimental parameters for the FIDCSI sequence are: FOV= 240x360 mm^2, slice thickness= 30 mm, 8x8 phase encoding steps (nominal voxel volume= 40.5 cm^3), TR=2,000 ms, complex data points=1,024, spectral bandwidth=5.0 kHz, pre-acquisition delay= 1.7ms, and NEX=16. The FIDCSI sequence in the ^{1}H mode was used to shim on the axial slice priori to the ^{31}P measurement.

To optimize the right and left voxel positions for the PF and BG regions, the 8x8 CSI grid was shifted accordingly prior to the IFT. The remaining post-processing and quantification steps were 100% automated. With a 5Hz Gaussian apodization, the ^{31}P resonances, phosphomonoester (PME), phosphodiester (PDE), phosphocreatine (PCr), adenosine triphosphate (γ-, α- and β-ATP), and inorganic orthophosphate (Pi), were modeled in the time domain with Gaussian damped sinusoids and by omitting the first 2.75ms of the FID using the Marquardt-Levenberg algorithm. Additionally, the relatively broad peaks underlying the PDE resonances [PME(i-τ_c)+PDE(i-τ_c)], which are due to less mobile molecules with PDE and PME moieties (e.g., synaptic/transport vesicles and micelles, and phosphorylated proteins), were quantified by taking the difference between the total modeled amplitude when omitting 3.6ms and 0.6ms of the FID (1). This approach ensured that the quantified PME and PDE primarily reflected the freely mobile MPL precursors [PME(s-τ_c)] and breakdown products [PDE(s-τ_c)], respectively (1).

A generalized linear regression model (SAS Institute Inc., PROC GENMOD) with subject group, age and hemisphere as the main effect terms was used to test bilateral group differences in each region. A second model with an additional subject group-by-hemisphere interaction term was used to test lateral group differences.

RESULTS

In the PF, the subject-by-hemisphere interaction was significant for PME(s-τ_c) and PDE(s-τ_c) levels (p=0.049 and p=0.034), and PCr/Pi ratios (p=0.0053). The differences of least squares results, showed decreased right PME(s-τ_c) and right PCr/Pi in ADHD children compared to controls (p=0.0034 and p<0.0001), while the lower right PDE(s-τ_c) levels failed to reach significance (p0.053). Additionally, in the PF the β-ATP levels were bilaterally higher in the ADHD children compared to controls (p=0.045).

In the BG, the only group difference was a bilateral PME(s-τ_c) reduction in the ADHD children compared to controls (p=0.029).

There were no lateral/bilateral group differences in PCr or PME(i-τ_c)+PDE(i-τ_c) levels, or PME(s-τ_c)/PDE(s-τ_c) ratios in either regions.

DISCUSSION AND CONCLUSIONS

The decreased PME(s-τ_c) suggests decreased synthesis of MPL in the right PF and bilaterally in the BG. With the trend of lower breakdown products of MPL in the right PF and the lack of a group difference in the equilibrium of MPL turnover [i.e., PME(s-τ_c)/PDE(s-τ_c) ratios], suggest reduced membrane mass or content, which is consistent with a lack of development or an underdevelopment of neuronal processes and synapses in ADHD. Additional support for this lack of neuronal development in ADHD is provided by the decreased utilization of high-energy phosphate metabolism in the right PF.

These alterations are, in part, consistent with structural MRI data, which in genneral show overall smaller brain volume and more pronounced differences on the right side. Additionally, these results suggest either a lack of development or an underdevelopment of neuronal processes and synapses in regions that are involved in the function of attention.

1. Stanley, JA, Pettegrew, JW, Magn Reson. Med., 45, 390-396, 2001.