Alterations of Membrane Phospholipid Precursor Levels in Treated and Stimulant-Naive Children with Attention-Deficit/Hyperactivity Disorder (ADHD)

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INTRODUCTION

The neurodevelopmental disorder, attention-deficit/hyperactivity 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. In a preliminary *in vivo* phosphorus (^{31}P) spectroscopy study [a noninvasive technique that can directly assess the metabolism of membrane phospholipids (MPL) and high-energy phosphates in multiple brain regions], we have shown deficits in MPL precursor levels in the basal ganglia (BG) and prefrontal (PF) regions of treated children with ADHD compared to healthy control subjects (1). These alterations reflect a deviation of normal brain development and are consistent with structural MRI data showing smaller brain volumes in children with ADHD. The purpose of this study is to assess possible molecular/biochemical alterations in relatively younger children with ADHD who are stimulant-naïve compared to control subjects. We hypothesize similar MPL metabolite deficits in the PF and BG regions of the younger cohort consistent with an underdevelopment of neuronal processes and synapses in ADHD.

SUBJECTS AND METHODS

Fifteen stimulant-naïve children with DSM-IV ADHD (nADHD; all males; mean age 8.1±1.3 years; age range 6.3 to 10.0 years; 9 with the combined type and 6 with the predominantly inattentive type), 9 treated ADHD children [tADHD; all males; mean age 10.1±1.3 years; age range 8.3 to 11.9 years; 6 with the combined type and 3 with the predominantly inattentive type; 8 ADHD children refrained from taking the stimulant medication at least 24-hours prior the scan; presented in part in (1)] and 17 healthy control subjects (HC; all males; mean age 9.2±1.9 years; age range 6.4 to 12.1 years) participated in this study.

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 3.2ms 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 3.2ms and 0.2ms of the FID (2). 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 (2).

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 as well as a third model to address group-by-age interactions were used.

RESULTS

Combined ADHD subjects vs HC: In the PF, independent of hemisphere PME(s-τ_c) levels were significantly lower in the conbined ADHD subject compared to HC (p=0.019) and there was a significant group-by-age interaction with PME(s-τ_c) levels (p=0.043), which showed lower levels in the older ADHD subjects. There was a significant age correlation with PME(s-τ_c) in the PF of ADHD subjects (r=-0.43; p=0.015), which was not present in the HC subjects. Additionally, there were no significant differences in the metabolite levels [including PME(i-τ_c)+PDE(i-τ_c) levels] in the BG.

nADHD, tADHD and HC: In the PF, the group term was significant for PME(s-τ_c) (p=0.027) and post-hoc analysis showed significantly lower PME(s-τ_c) levels in the tADHD compared to HC subjects (p=0.0012). In the BG, the group term failed significanse; however, the differences of least-squares means showed significantly lower PME(s-τ_c) levels in the tADHD compared to HC subjects (p=0.022). Also, age was significantly different between nADHD and tADHD (p=0.0018).

DISCUSSION AND CONCLUSIONS

Overall, there is a significant deficit in the MPL precursor levels in the PF of the combined ADHD subjects as well as a precursor level-age association suggesting greater precursor level deficits in the relatively older ADHD children (and suggestive of being independent of treated vs stimulant-naïve status). In the BG, the MPL precursor deficits appear more dominant in the tADHD subjects. These deficits suggest decreased synthesis of MPL and as a result reduced membrane mass or content, which is consistent with a lack of development or an underdevelopment of neuronal processes and synapses in ADHD. However, the sample size of each subgroup is limited and is unclear if these effects are truly due to stage of brain maturation or treatment effect. Additionally, further investigation is needed to address possible symptomatology effects.

1. Stanley, JA, et al., Society for Neuroscience (800.11), 2004.
2. Stanley, JA and Pettegrew, JW, Magn Reson. Med., 45, 390-396, 2001.