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EVIDENCE OF ALTERED ENERGY METABOLISM IN AUTISTIC CHILDREN

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Abstract

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1. In this pilot study, the authors investigated the hypotheses there are increased concentrations of lactate in brain and plasma and reduced brain concentrations of N-acetyl-asparate (NAA) in autistic children.
2. NAA and lactate levels in the frontal lobe, temporal lobe and the cerebellum of 9 autistic children were compared to 5 sibling controls using MRS. Plasma lactate levels were measured in 15 autistic children compared to 15 children with epilepsy.
3. Preliminary results show lower levels of NAA cerebellum in autistic children (p=0.043). Lactate was detected in the frontal lobe in one autistic boy, but was not detected any of the other autistic subjects or siblings.
4. Plasma lactate levels were higher in the 15 autistic children compared to 15 children with epilepsy (p=0.0003).
5. Higher plasma lactate in the autistic group is consistent with metabolic changes in some autistic children. The findings of altered brain NAA and lactate in autistic children suggest that MRS may be useful characterizing regional neurochemical and metabolic abnormalities in autistic children.

Keywords: autism, lactate, N-acetyl-asparate, magnetic resonance spectroscopy

Abbreviations: N-acetyl-asparate (NAA), magnetic resonance spectroscopy (MRS), nicotinamide dinucleotide (NAD)

Introduction

There are now several lines of evidence pointing to abnormalities of serotonergic neurotransmission in autism. The first suggestion that serotonin was altered in autism was in a study by Schain and Freeman (1961) reporting increased platelet serotonin in approximately one-third of autistic patients. Recently, a study of serotonin synthesis using positron emission tomography with the tracer α[C-11]methyl-L-tryptophan directly demonstrated altered serotonin synthesis in the brain in autistic boys (Chugani et al., 1997). Further, genetic studies have implicated the serotonin transporter in autism (Cook et al., 1997).

There is also evidence for a role of the immune system in the pathophysiology of a subset of autistic patients (Warren, 1998). The authors asked how immune activation in some autistic children might be linked to serotonergic abnormalities in autism. Since serotonin synthesis is limited by precursor concentration, decreased delivery of tryptophan, the precursor for serotonin synthesis, to the brain results in a decrease in serotonin content (Fernstrom and Wurtman, 1971). Immune stress or infection results in decreased plasma tryptophan due to induction of tryptophan metabolism in macrophages by indoleamine 2,3-dioxygenase (Saito et al., 1993). *Tryptophan depletion by this mechanism may represent an immune mechanism involved in the pathophysiology of autism, or may represent a mechanism of exacerbation of autistic symptoms during immunological stress.* In fact, experimentally induced tryptophan depletion has been shown to exacerbate autistic symptoms in autistic adults (McDougle et al., 1996). In addition, decreased plasma tryptophan levels might also result in decreased production of nicotinamide dinucleotide (NAD) in the liver by tryptophan 2,3-dioxygenase. A decrease in NAD would result in decreased electron transport in the mitochondria and might be expected to result in increased lactate concentration in plasma and brain, and be related to the hypotonia described in many autistic children.

In order to test this hypothesis, the authors measured lactate in the plasma in of a group of autistic children who participated in a positron emission tomography study of serotonin synthesis and compared values to a group of epileptic children who were studied using the same protocol. Lactate was measured in brain with MRS. Lactate, not detectable in normal brain by MRS (Frahm and Hanefeld, 1996; Moore, 1998), has been detected in with MRS in brain in a number of disorders (Barkovich et al., 1993; Barker et al., 1994; Jenkins et al., 1993; Ashwal et al., 1997). In addition, N-acetyl-aspartate (NAA), a putative neuronal marker that is an indicator of neuronal function/viability and has been implicated in the neuro-pathophysiology of several disease states, was measured.

Methods

Subjects.

For the MRS studies, 9 autistic children (8 males, 1 female, ages 3-12 years, mean age = 5.7 years $\pm$ 2.5 SD) and 5 of their siblings (4 males, 1 female, ages 6-14 years, mean age =9 years $\pm$ 3 SD) were recruited from clinics at the Children's Hospital. For the plasma lactate studies, 15 autistic children (12 males, 3 females, mean age 5.1 years $\pm$ 1.8 SD) were compared to 15 children with epilepsy (11 males, 4 females, mean age 6.1 years $\pm$ 4.9 SD). Studies were performed in compliance with regulations of Wayne State University Human Investigation Committee, and written informed consent of parent or guardian was obtained prior to all studies. In addition, written assent was obtained from the siblings over 8 years of age.

Children were included in the autistic group only if they met all of the following criteria: (1) DSM-IV (Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition) criteria for autism based on unstructured interview; (2) autism quotient greater than 85 on Gilliam Autism Rating Scales; (3) scores higher than 30 on Childhood Autism Rating Scales; (4) previous diagnosis by a treating neurologist or psychiatrist. They were excluded from the sibling or epilepsy groups if they met any of the above 4 criteria. Studies were performed in compliance with regulations of Wayne State University Human Investigation.

MRS Measurements.

MRS studies were performed on a clinical 1.5T MRI/MRS system (Horizon 5.6, General Electric, Milwaukee) using a STEAM technique (TE = 30 msecs, TM = 13.7 msecs, TR = 2000 msecs). In vivo proton MRS spectra were acquired from frontal lobe, temporal lobe and cerebellum volumes of interest (8 cc) as shown in Fig. 1, with an acquisition time of 5 minutes per region. Compounds which were identified in the short echo 1H MRS were the neuronal marker NAA and lactate. The area under each of the resonances is proportional to the concentration of the specific neurochemical compound. Individual peak areas were fit using time domain analysis software (deBeer et al., 1992; van den Boogaart et al., 1994) and the concentrations of each compound are reported in arbitrary quantitative units as a ratio to brain water concentration ($x10^4$/water). This water referencing method has been used in the field for over a decade and has been validated by a number of research groups (Thulborn and Ackerman, 1983; Frahm et al., 1990; Klose, 1990; Christiansen et al., 1993; Hetherington et al., 1996; Soher et al., 1996). The analysis software is public domain (http://carbon.uab.es/mruiwww) and eliminates much of the subjectivity previously involved in determining spectral peak areas using older methods. Statistical comparison between the two groups was performed using a one-tailed t-test.

Fig 1. T2-weighted axial magnetic resonance imaging scans demonstrating the locations of the volumes of interest used for spectroscopy measurements.

Plasma Lactate Measurements.

Subjects were fasted after 8 a.m. and blood was drawn between 2 and 3 p.m. Blood was collected into a tube containing heparin and was immediately centrifuged. Plasma was stored at -70°C until lactate measurements were made. Lactate was measured using Sigma kit No. 735 (St. Louis, MO).

Data Analysis.

Statistical comparison between the two groups was performed using a two-tailed independent groups t-test.

Results

MRS Measurements.

Lactate was detected in the frontal cortex in one autistic subject (Fig. 2). Lactate was not detected in any of the other autistic or control subjects. NAA was significantly lower in cerebellum in the autistic group than in the sibling group ($p=0.043$), while NAA values for frontal lobe and temporal lobe did not significantly differ between the groups (Fig. 3).

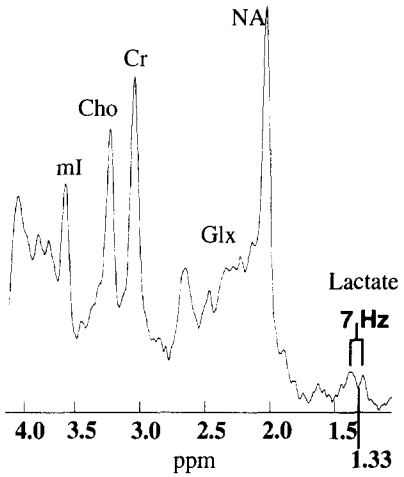

Fig 2. Proton spectra from the frontal lobe in a 7 year old autistic boy. (mI = myo-Inositol, Cho = choline, Cr = creatine/phosphocreatine, Glx = glutamine/glutamate/GABA, NA = Nacetyl-aspartate).

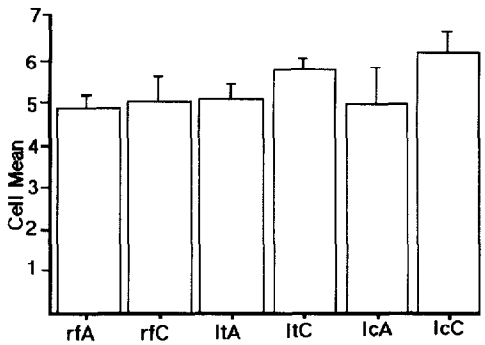

Fig 3. Regional concentrations of N-acetyl-aspartate. Bars represent the mean and error bars display the standard error of the mean. (rfA = right frontal/autistic group, rfC = right frontal/control group, ltA = left temporal/autistic group, ltC = left temporal/control group, lcA = left cerebellum/autistic group, lcC = left cerebellum/control group)

Plasma Lactate Measurements.

Plasma lactate levels were significantly higher in the autistic group (mean 15.3 mg/dl $\pm$ 3.1 SD) than in the epileptic group (mean 10.9 mg/dl $\pm$ 1.9 SD) ($p = 0.0003$).

Discussion

These results, although preliminary, demonstrate higher plasma lactate in autistic children. Furthermore, lactate, which is not detected in normal brain, was detected in 1 of the 9 autistic children studied, but in none

of the controls. These data are consistent with altered energy metabolism in some autistic children, perhaps due to decreased NAD production. A possible therapeutic approach to treat a relative lack of NAD might be to supplement with niacin or to use compounds which have been used to improve mitochondrial function. The most frequently used substance to be used to improve mitochondrial function in humans (including children) with mitochondrial cytopathies is coenzyme Q10 (Ogasahara et al., 1986; Yamamoto et al., 1987; Bresolin et al., 1988; Ihara et al., 1989; Abe et al., 1991; Bendahan et al., 1992; and Heb et al., 1993). Following oral administration of conenzyme Q10 in patients with MELAS, decreases in CSF lactate and pyruvate (Ihara et al., 1989; Abe et al., 1991), decreases in serum lactate and pyruvate (Yamamoto et al, 1987), and clinical improvement in these studies. Similarly, coenzyme Q10 treatment resulted in decreased serum lactate pyruvate, improved mitochondrial function in platelets and increased muscle strength in patients with Kearns-Sayre syndrome (Bresolin et al., 1988; Ogasahara et al., 1986). Furthermore, coenzyme Q10 reduced brain lactate concentrations in patients with Huntington's Disease (Koroshetz et al. 1997), a disease in which excitotoxicity through NMDA receptor stimulation has been postulated.

Conclusion

This study demonstrates evidence of altered energy metabolism in some autistic children. The finding of lower NAA in cerebellum in the autistic group is consistent with neuropathology reports of decreased numbers of Purkinje cells and granule cells in cerebellar cortex from autistic individuals (Bauman and Kemper, 1994). Although the sample sizes are small, the present study suggests further MRS studies in autistic groups are warranted.

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