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Reduction in Glutamine/Glutamate Levels in the Cerebral Cortex after Adrenocorticotropic Hormone Therapy in Patients with West Syndrome

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West syndrome (WS), an intractable epileptic encephalopathy of infancy, is refractory to many antiepileptic drugs; however, adrenocorticotropic hormone (ACTH) is an effective treatment for WS. The mechanism behind the efficacy of ACTH is mediated by biochemical processes that remain unknown. We examined the effects of ACTH therapy with tetracosactide (TCS), a synthetic ACTH analogue, on brain metabolism in patients with WS, using ^{1}H magnetic resonance spectroscopy (^{1}H-MRS). In six patients with cryptogenic WS, we performed single-voxel ^{1}H-MRS at the occipital lobe cortex. Measurements were taken prior to TCS treatment, a few days after therapy, and several months after therapy. Data were also compared with subjects having only mild psychomotor delays. The metabolites measured were glutamine plus glutamate (Glx), N-acetylaspartate (NAA), choline (Cho), and myoinositol (mI); each was expressed as a ratio with creatine plus phosphocreatine (total creatine: tCr). The Glx/tCr ratio was significantly reduced after the TCS treatment. The NAA/tCr ratio was also significantly reduced after the treatment compared with the control group, although the change in NAA signal was heterogeneous among patients, correlating with respective outcomes. The Cho/tCr and mI/tCr ratios were not affected by TCS treatment. The reduction in Glx suggests a decrease in the glutamate-glutamine cycle, which plays a pivotal role in synthesizing neurotransmitters such as glutamate and GABA. TCS-induced Glx reduction may induce changes in synaptic signal transduction, thereby accounting for the effect of TCS on WS. The change in NAA indicates altered neuronal activity, which may be correlated with outcome in WS patients.

Keywords: adrenocorticotropic hormone; glutamine-glutamate cycle; ^{1}H magnetic resonance spectroscopy; tetracosactide; West syndrome
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Introduction

Therapy with ACTH or its synthetic analogues has been a mainstay of treatment for patients with West syndrome (WS), which is an intractable epileptic encephalopathy of infancy and early childhood (Shumiloff et al. 2013). The mechanism of action of ACTH in WS is thought to be unique compared with other antiepileptic drugs. In experimental conditions, a single injection of ACTH does not prevent a seizure, whereas most antiepileptic drugs have been shown to have direct neuroinhibitory effects. Repeated administration of ACTH, however, will exert antiepileptic effects, accompanied by changes in cerebral metabolism, parts of which may be involved in cessation of spasms (Pranzatelli 1994; Tekgül et al. 1999; Edwards et al. 2002).

Magnetic resonance spectroscopy (MRS), a noninvasive method of measuring metabolic conditions *in vivo*, has demonstrated ACTH-related metabolic alterations in the brain. A study with ^{31}P-MRS showed that ACTH treatment decreased phosphomonoesters (Yoshioka et al. 1994), and ^{1}H-MRS demonstrated that N-acetylaspartate (NAA) signals were reduced during ACTH therapy (Maeda et al. 1997). These reports showed that ACTH affects cerebral biochemical processes. The aim of the present study was to elucidate cerebral metabolic changes after ACTH therapy in patients with WS by using ^{1}H-MRS with a short echo time to examine levels of glutamine plus glutamate (Glx) and myoinositol (mI), as well as levels of NAA and choline

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(Cho).

Patients and Methods

We studied six patients with WS who had been treated with ACTH therapy. All subjects exhibited spasms in clusters and hypsarrythmia on electroencephalography (EEG). They showed no malformations based on clinical MRI studies. No abnormalities detectable by screening for inborn metabolic errors (blood gas, amino acids, organic acids and lactate/pyruvate) and congenital infections were observed. Patient data are summarized in Table 1. The objectives of this study were explained to the families of patients, and informed consent was obtained. The study protocol was approved by the Ethics Committee of Tohoku University School of Medicine.

Since WS patients can occasionally respond to vitamin B6 or antiepileptic drugs, vitamin B6 (30 mg/kg/day for 1 week) and then valproate (VPA; 30 mg/kg/day for less than 2 weeks) were initially tested. When these medicines were found to be ineffective, ACTH therapy was initiated as follows: tetracosactide acetate (TCS; Cortrosyn, Daiichi Pharmaceutical Co., Tokyo), a synthetic ACTH analogue, was administered intramuscularly daily for 2 weeks, at a dosage of 0.015-0.020 mg/kg, and subsequently for a week at the same dosage on alternate days. Finally, three additional halved doses were given on alternate days.

^{1}H-MRS was obtained when clinically necessary MRI studies were performed at Takuto Rehabilitation Center for Children using a 1.5-T magnetic resonance (MR) unit (MAGNEX ECLIPSE; Marconi Medical Systems, Cleveland, OH, USA). The first ^{1}H-MRS was performed at 1-32 days (mean of 11 ± 12 days) before the start of TCS treatment. Subsequently, a second ^{1}H-MRS was performed at 2-9 days (mean of 4 ± 3 days) after TCS treatment ("soon after" values), and a third ^{1}H-MRS was performed at 6-11 months (mean of 9 ± 3 months) after the end of TCS treatment ("long after" values). A single-voxel ^{1}H spectrum was obtained from a 1.5 × 1.5 × 1.5-cm volume of interest (VOI) that was set at the same position, aimed at the cuneus in the occipital lobe cortex, for all measurements. All spectra were obtained with a point-resolved spectroscopic (PRESS) sequence at an echo time (TE) of 35 ms. The repetition time (TR) was 1,500 ms. After global and local shimming and optimization of the water-suppression pulse, data were collected 192 times and averaged.

Spectral processing and fitting were automatically performed using a program incorporated into the workstation software for the MRI unit (VIA2.0, Marconi Medical Systems). The fitting for resonance signals was based on the LC Model (linear combination of model spectra of metabolite solutions *in vitro*) (Provencher 1993), which is a user independent, frequency-domain fitting routine. Major resonance peaks in the spectra were noted at a TE of 35 ms: N-acetyl-containing compounds (primarily NAA) at 2.0 ppm, glutamate plus glutamine (Glx) at 2.1-2.5 ppm, choline-containing compounds (Cho) at 3.2 ppm, myoinositol (mI) at 3.6 ppm, and creatine plus phosphocreatine (designated as total creatine or tCr) at 3.0 ppm. Signal intensities were measured by calculating the area of the peak. Given that tCr is ubiquitously distributed and relatively stable during postnatal maturation of the brain, even in pathologic conditions (Castillo et al. 1996), and that it does not change during ACTH therapy (Maeda et al. 1997), the signal intensities were expressed relative to the tCr signal.

Control groups were constructed by adding ^{1}H-MRS sequences to clinical diagnostic MRI in non-epileptic cases with mild psychomotor developmental delays without abnormalities on conventional MRI and standard metabolic screening. To control for metabolite signal changes with cerebral development (Pouwels et al. 1999), two age-matched control groups were constructed, one for comparison with the results before and soon after TCS treatment (designated as the pre-control group, age 9 ± 2 months, n = 7) and one group for comparison with the results long after therapy (designated as the after control group, age 18 ± 5 months, n = 9). In each patient, the VOI was set as near as possible to the same position within the occipital lobe as for the patients.

The changes in Glx/tCr, NAA/tCr, Cho/tCr and mI/tCr ratios in MRS data from the patients along with TCS treatment were analyzed using the Friedman test. The ratios between patient and control groups were compared using the Mann-Whitney test.

Results

All patients completed TCS treatment without serious complications. The tonic spasms were eliminated and electroencephalographic hypsarrythmia disappeared in all patients, although partial seizures and generalized seizures emerged after the end of the therapy in patients 5 and 6, respectively. In patients 2 and 3, AED was continued after TCS treatment because epileptic spikes were observed. One year after the end of TCS treatment, patient 2 showed normal psychomotor development and mild to severe developmental delay remained in the other five patients (Table 1).

Fig. 1A shows serial MRI images obtained before (a), soon after (b), and long after (c) TCS treatment (patient 2). The white squares in the occipital lobe represent the VOI for ^{1}H-MRS measurements. As shown, diffuse brain shrinkage was observed on MRI soon after TCS treatment. Fig. 1B shows ^{1}H-MRS serial spectra obtained before (a), soon after (b), and long after (c) TCS treatment at VOIs corresponding to those in Fig. 1A. At the short echo time, the signals for NAA, Glx, tCr, Cho and mI were detected. Soon after ACTH therapy, the Glx and NAA signals were reduced relative to the tCr signal. Long after the therapy, the Glx and NAA signals increased again.

Fig. 2 shows the results of serial measurements of the metabolite signal intensity ratio relative to tCr for Glx, NAA, Cho and mI in WS patients. Data from the control groups are also plotted. The results of the measurements for the Glx/tCr ratio are shown in Fig. 2A. The ratio before treatment was not significantly different from that of the control group. After TCS treatment, the ratio was significantly reduced to a level well below that of the control group. The Glx/tCr ratios in patients had recovered to control levels long after therapy. As shown in Fig. 2B, the NAA/tCr ratios were significantly lower than those in the control group, although the change was heterogeneous among patients. The NAA/tCr ratios before and long after therapy did not differ from those of the respective control groups. The Cho/tCr ratio tended to decrease during the course of measurements, although the ratios before and long after TCS treatment did not differ from those of the respective control groups (Fig. 2C). The mI/tCr ratio showed no statistically significant changes (Fig. 2D).

Table 1. Clinical features of study patients.

Patient No./sex	Gestational age at birth (weeks)	Age (months)		Antiepileptic drugs		State of epilepsy at ^{1}H-MRS				Psychomotor development
		at seizure onset	at first ^{1}H-MRS	Before TCS treatment	After TCS treatment	SA		LA		
						Sp.	Sz.	Sp.	Sz.	
1/F	40	10	10	VPA	−	−	−	−	−	moderate
2/F	40	4	4	VPA	NZP	+	−	+	−	normal
3/M	40	5	7	VPA	NZP	+	−	+	−	severe
4/F	39	8	9	VPA	−	−	−	−	−	mild
5/F	40	8	8	VPA	ZNS	+	+	+	+	moderate
6/F	35	3	4	VPA	ZNS	+	+	+	+	severe

M, male; F, female; ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; TCS, tetracosactide (a synthetic ACTH analogue); VPA, valproate; NZP, nitrazepam; ZNS, zonisamide; Sp., epileptic spike on electroencephalography; Sz., epileptic seizure; SA, soon (4 ± 3 days) after TCS treatment; LA, long (9 ± 3 months) after TCS treatment. Psychomotor development was evaluated using the Enjoji Development Test at 1 year after TCS treatment and is represented as normal (developmental quotient: 75-100), mild (50-74), moderate (35-49), or severe (< 34).

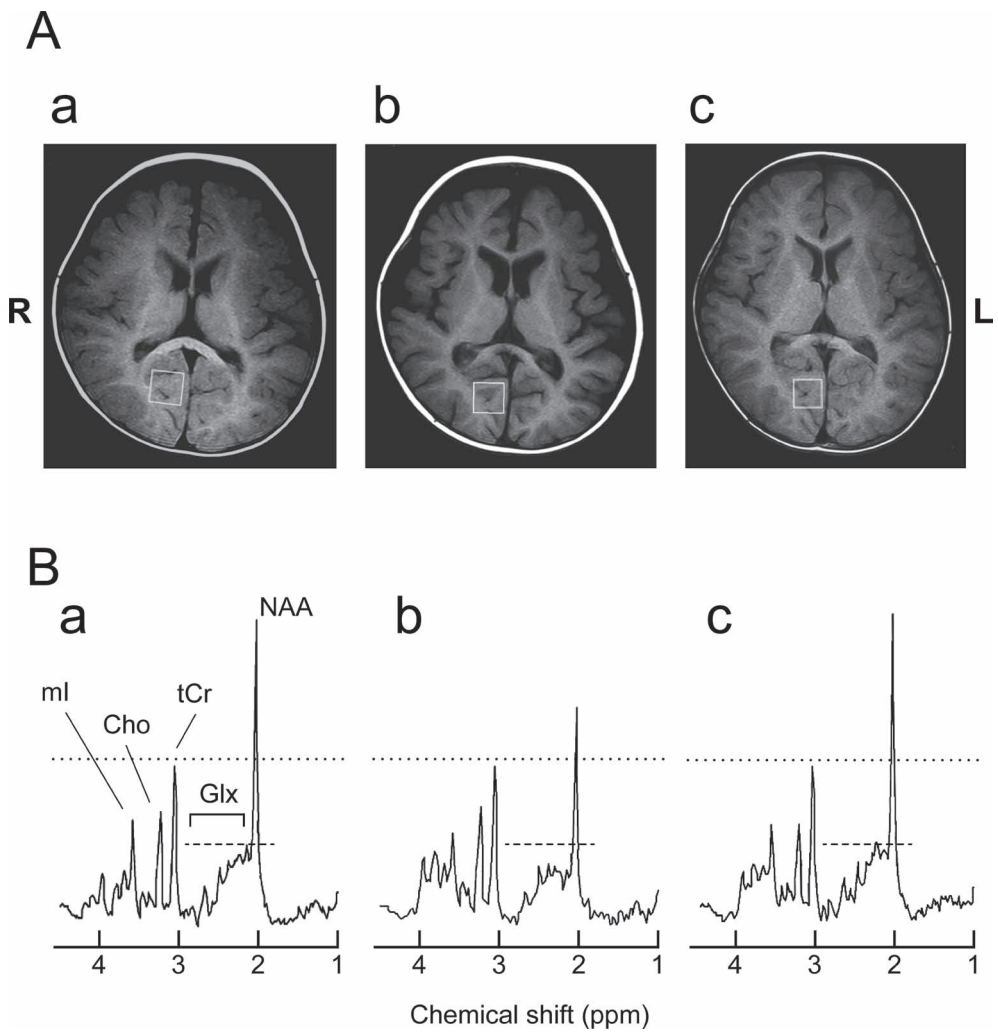

Fig. 1. Changes in MRI and ^{1}H-MRS spectrum according to TCS treatment. MRI and ^{1}H-MRS were performed before, soon after and long after TCS treatment. A. T1-weighted images before (a), soon after (5 days) (b) and long after (10 months) (c) TCS treatment (patient 2). White squares indicate the location of the volume of interest (VOI) for ^{1}H-MRS. R, right; L, left. B. ^{1}H-MRS results before (a), soon after (b), and long after (c) TCS treatment at VOI corresponding to those in Fig. 1A. NAA, n-acetylaspartate; Glx, glutamine plus glutamate; tCr, creatine plus phosphocreatine; Cho, choline; mI, myoinositol. The vertical scale was normalized to the tCr signal (dotted line). Broken lines indicate the Glx level before TCS treatment.

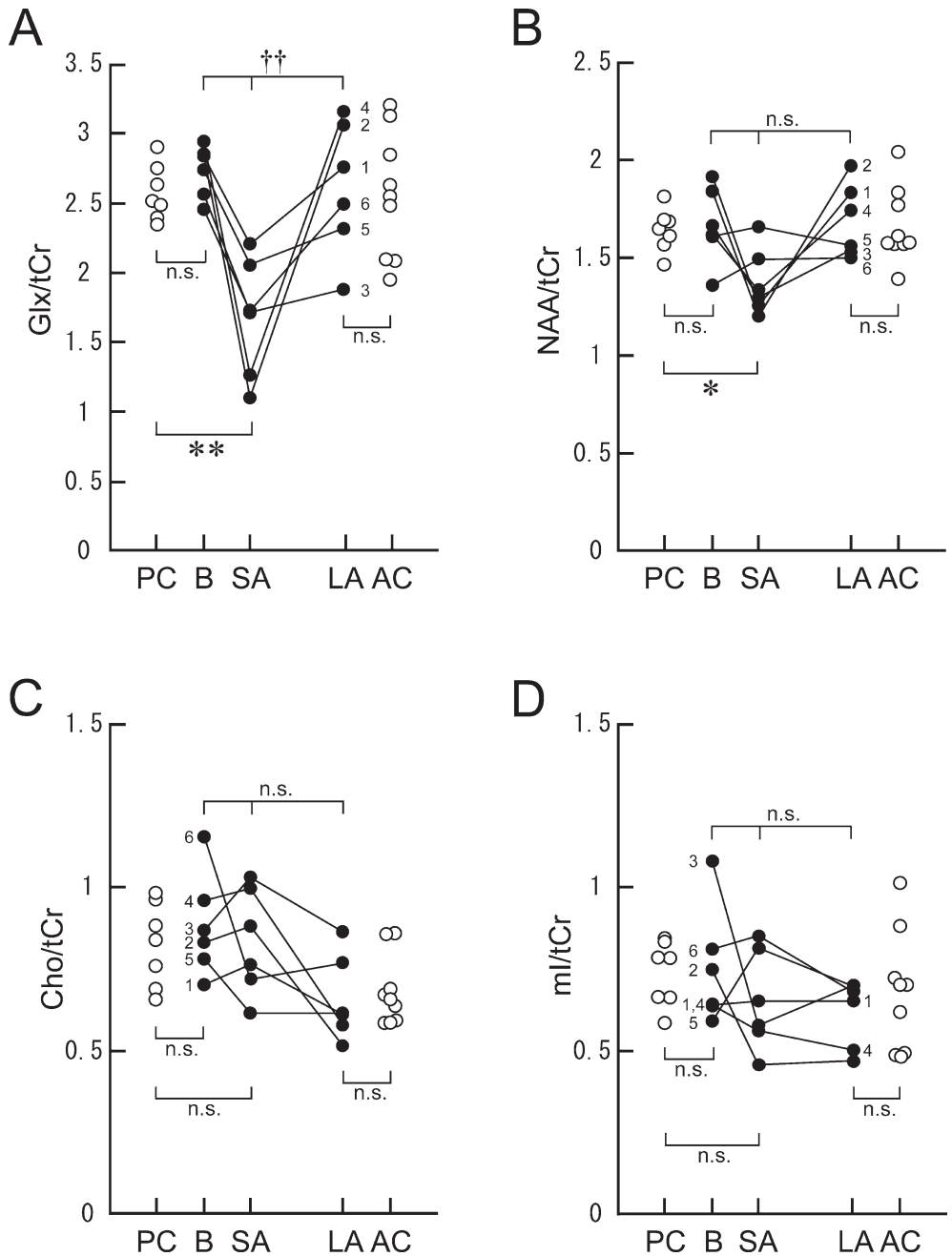

Fig. 2. Summarized brain metabolite changes in ^{1}H-MRS according to TCS treatment. Glx/tCr (A), NAA/tCr (B), Cho/tCr (C), and mI/tCt (D) ratios are plotted. Open circles and filled circles are plots for controls and patients, respectively. Lines connect the data from each patient. Numbers beside the filled circles indicate the patient's number corresponding to those in Table 1. PC, pre-control; B, before TCS treatment; SA, soon after TCS treatment; LA, long after TCS treatment; AC, after control. † and †† represent statistical significance determined by Friedman test ($p < 0.05$ and $p < 0.01$, respectively). * and ** represent statistical significance determined by Mann-Whitney test ($p < 0.05$ and $p < 0.01$, respectively). n.s.: not significant.

Discussion

When ACTH is administered, multiple biochemical changes occur within the developing brain, including altered amino acid composition, reduced synthesis of corticotrophin-releasing hormone, an increase in neurosteroid synthesis, and reduced generation of neurotrophic factor (Pranzatelli 1994; Tekgül et al. 1999; Edwards et al. 2002; Brunson et al. 2002; Kokubo et al. 2002). Although the mechanism accounting for the efficacy of ACTH against WS remains to be determined, ACTH-induced biochemical changes are conceivably involved in cessation of epileptic spasms. In the present study using short-TE ^{1}H-MRS, we observed ACTH-induced metabolic changes that remained

after the withdrawal of ACTH, which may relate to its efficacy.

The Glx signal comprises signals from glutamate and glutamine. Glutamate is a major excitatory neurotransmitter in the CNS (Sheng and Kim 2002) that is virtually confined to neurons, while glutamine is mainly distributed in the glia (Kanamori et al. 1995). Glutamate and glutamine are tightly coupled in a glioneuronal metabolic cycle. Glutamate is synaptically released from glutamatergic neurons, taken up by astroglial cells, and converted to glutamine, which is converted back to glutamate in neurons (Fig. 3; Sibson et al. 1998). This glutamate-glutamine cycle is a major pathway for maintaining synaptic glutamate. In addition, production of γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter derived from glutamate ("GABA shunt"), also depends on the cycle (Liang et al. 2006). Maintenance of this glutamine-glutamate cycle accounts for approximately 70-80% of the total energy consumption within the cerebral cortex (Erecinska and Silver 1990). As Glx consists of signals from glutamine and glutamate, it corresponds to the glutamine-glutamate cycle (Castillo et al. 1996).

After the TCS treatment, the Glx/tCr ratio was decreased beyond that of the non-epileptic controls, implying that the reduction of Glx was not secondary to ameliorated epileptic activity. Instead, the reduction may be the result of TCS effects on the glutamate-glutamine cycle. This cycle is closely related to glutamatergic synaptic density and activity in cerebral tissue (Ottersen et al. 1992; Woermann et al. 2001). Histological studies have reported that systemic increases in ACTH result in transient atrophy of the apical dendrites of rat pyramidal neurons (Ohta et al. 1982). The atrophy may be mediated by the upstream effector corticotrophin-releasing hormone, because the direct application of ACTH does not produce such an effect in vitro (van der Neut et al. 1992). Alternatively, a decrease in glutamine induced by the catabolic effects of ACTH remains a possibility (Newsholme et al. 2003). As apical dendrites are major sites of glutamatergic synaptic input for cortical pyramidal neurons (Douglas and Martin 1998), dendritic atrophy implies a reduction in excitable connectivity among cortical neurons (Ábrahám et al. 1996). The decrease in Glx caused by TCS may reflect such a reduction in the glutamatergic synaptic strength. Because synaptic GABA release is regulated by the glutamate-glutamine cycle (Liang et al. 2006), GABAergic synapses may also be affected by TCS. Although the decrease in Glx was temporary, it may modify synaptic connectivity between neurons during the age range when patients are vulnerable to WS.

The Glx signal can increase after prolonged seizures (Neppl et al. 2001). In the current study, however, Glx in WS patients before ACTH therapy did not differ from that in non-epileptic controls, implying that hypsarrythmia and daily spasms did not alter Glx. All WS patients received VPA at the time of the ^{1}H-MRS measurement before ACTH therapy. Continuous VPA treatment has been reported to increase Glx (Petroff et al. 1999; Garcia et al. 2009). The short duration of VPA treatment (less than 2 weeks) until the initial ^{1}H-MRS measurement may explain why Glx did not increase despite VPA in the present study.

NAA synthesis is coupled with neuronal energy metabolism and considered a marker for neuronal activity (Moffett et al. 2007). NAA and the glutamate-glutamine cycle are indirectly linked by the Krebs cycle (Fig. 3). NAA signals contain relatively weak signals from N-acetylaspartylglutamate (NAAG), which is also linked to the glutamate-glutamine cycle. However, it is unknown whether reduction in the net NAA signal is affected by the TCS-induced changes in the glutamate-glutamine cycle. Conversely, biochemical studies revealed that ACTH reduces glutathione levels (Lach et al. 1986), which may

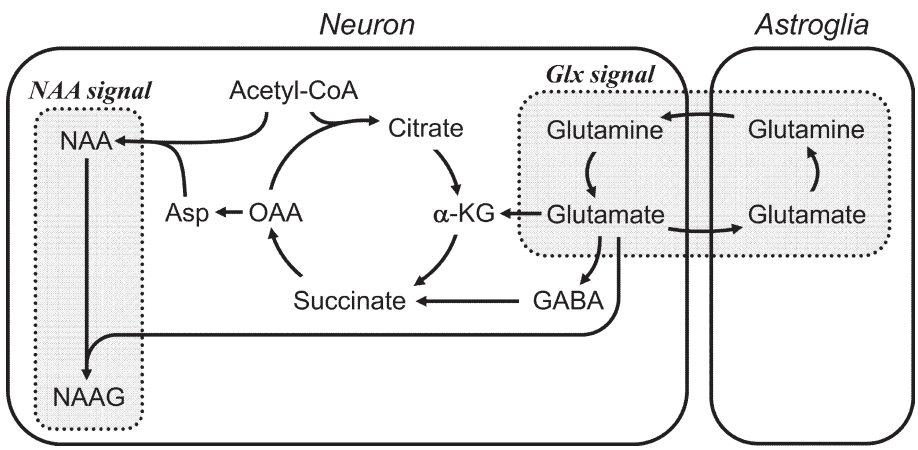

Fig. 3. Metabolic relationship between Glx, NAA and related compounds. Synaptically released glutamate is taken up by astroglia and converted to glutamine. Glutamine is then transferred from astroglia to neurons and converted to glutamate, thereby completing the glutamate-glutamine cycle. Since the Glx signal in ^{1}H-MRS consists of signals predominantly from glutamate and glutamine, Glx corresponds to the amount of glutamate-glutamine cycling. The NAA signal comprises signals from NAA and N-acetylaspartylglutamate (NAAG). Asp, aspartate; GABA, γ-aminobutyric acid; α-KG, α-ketoglutarate; OAA, oxaloacetate.

cause a reduction in NAA synthesis (Heales et al. 1995). NAA is a major organic osmolyte of neurons (Taylor et al. 1995; Baslow 2003); thus, reduced NAA may result in neuronal volume reduction. This may partly underlie the transient brain shrinkage that occurs during ACTH therapy.

The change in the NAA signal was heterogeneous among the enrolled patients. In patients 1, 2, and 4, who had relatively good outcomes, the NAA signal decreased transiently soon after TCS treatment and then increased to relatively high values. In patients 5 and 6, who had epileptic seizures after TCS treatment, the NAA signal remained low throughout the ^{1}H-MRS measurements. In patient 3, who had severe psychomotor developmental delay, the NAA signal remained low for months after the TCS treatment. Therefore, the change in the NAA signal may be correlated with outcome in WS patients.

Cho signals correlate with cellular membrane turnover (Castillo et al. 1996). The Cho signal during the neonatal period is relatively high and is reduced during the course of development (Pouwels et al. 1999). In our WS patients, Cho signals tended to decrease over time but did not differ from those of controls, suggesting the gradual reduction during normal development. Although the Cho signal can be temporarily increased by prolonged epileptic phenomena (Lazeyras et al. 2000), signal intensities are not different from controls after the elimination of seizures.

The mI signal is confined to glial cells. This metabolite is involved in osmoregulation and in a second messenger system; it is also a possible precursor of glucuronic acid (Urenjak et al. 1993; Castillo et al. 1996). The amount of mI is reported to be reduced during the course of early cerebral maturation (Pouwels et al. 1999). In the present study, mI/tCr ratios tended to fall during the course of TCS treatment, although this change was not statistically significant. Reduced mI signals have been reported in patients taking VPA (Erecinska and Silver 1990), and mI increases have been seen in epileptic tissue accompanied by reactive gliosis (Wellard et al. 2003). In the present study, all patients tested received VPA before and soon after TCS treatment. However, the duration from the start of VPA medication to the first ^{1}H-MRS measurement was short, which may explain why mI did not decrease in this study.

Several limitations of this study should be considered. Since it was difficult to include a normal control group, the control data were obtained as part of clinical diagnostic MRI in non-epileptic cases with mild psychomotor developmental delays. The NAA/tCr ratio in patients with cognitive developmental delay tends to be lower than that in normal children, although the difference is not statistically significant (Hashimoto et al. 1995). Therefore, the NAA/tCr ratio in the controls could be lower than that in normal infants. Our clinical 1.5-T MRI system was unable to discriminate peaks from glutamate and glutamine. Further MRS investigation with a larger cohort using an advanced MRI system, which enables discrimination of signals from glutamate, glutamine and GABA, would facilitate understanding of the significance of changes in the glutamate-glutamine cycle during ACTH therapy.

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Ethical Publication

We confirm that we have read the Journal's position on ethical publication and affirm that this report is consistent with those guidelines.

Conflict of Interest

The authors declare no conflict of interest.

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