Neurometabolic Effects of ACTH on Free Amino Compounds in Opsoclonus-myoclonus Syndrome

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- paraneoplastic syndrome
- immunotherapy
- ataxia
- dancing eyes
- ACTH
- neuroblastoma

Abstract

To evaluate the possible role of central free amino compounds in pediatric opsoclonus-myoclonus syndrome (OMS), 21 cerebrospinal fluid (CSF) amino compounds were measured by an amino acid analyzer or mass spectroscopy in 74 anesthetized children, 54 with OMS and 20 age-matched neurological controls. In OMS, only phosphoethanolamine was increased compared to controls; OMS severity and duration had significant converse effects on alanine and phosphoethanolamine. In contrast, corticotropin (ACTH) treatment was associated with increased alanine and phenylalanine, and decreased taurine compared to controls and untreated OMS, and increased glutamine, lysine, ornithine, and tyrosine compared to untreated OMS. Other than low taurine, these effects were not found with corticosteroid treatment, and non-steroidogenic immunotherapy had no effect. The ACTH dose-association was most apparent for alanine and phosphoethanolamine, but lysine and ornithine were also higher in the high-dose ACTH group. There were no significant disease- or treatment-associated perturbations in GABA, glycine, or other amino acids. These data suggest a unique pattern of ACTH effects on non-neurotransmitter CSF amino compounds, for the most part not shared by steroids.

Introduction

Opsoclonus-myoclonus syndrome (OMS) is an autoimmune paraneoplastic disorder that affects individuals of all ages. In children, occult neuroblastoma of the chest or abdomen [37] triggers a devastating attack of the immune system on brain cells [29] through shared but unknown onconeural antigens. The patients precipitously lose their ability to talk, walk, or sleep normally, and become extremely irritable [13]. Long-term neurobehavioral sequellae are common [14].

We hypothesized an immune-mediated derangement in brain neurochemistry or neurotransmission to explain such striking neurological symptoms and signs, with the corollary that immunotherapy, which improves clinical symptoms, may help restore normal neurotransmission [26]. B- and T-cells, especially through autoantibodies and cytokines, are capable of attacking neurons and glia, thereby perturbing cellular metabolism, neural membrane function, synaptic activity, and neural networks [25]. Identifying a neurochemical imbalance could lead to new symptomatic treatments to improve neurological function. Amino acids are attractive candidates for study because they participate in brain metabolism as well as neurotransmission. Excitatory amino acids are intrinsic to the circuitry of many brain regions relevant to OMS, such as the cerebellum [7]. The inhibitory amino acid neurotransmitters GABA and glycine are the principal amino acids of known clinical significance to myoclonus, others being less well studied [24].

Amino acid measurement in cerebrospinal fluid (CSF) is reliable and has made a contribution to our understanding of brain neurochemistry in neurological disorders and neuropsychotrophic drug effects over the past two decades [36, 38]. Changes in CSF amino acid levels may reflect the severity of the underlying pathological state and may be useful in monitoring disease activity [34]. There has been no previous publication on amino acid measurements in CSF of children with OMS. We now report on 21 different CSF amino compounds in OMS. We evaluated the effect of adrenocorticotrophic hormone (ACTH), the "gold standard" of therapy, and corticosteroids, which also are commonly prescribed [25]. ACTH is more

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Table 1 Clinical information on subjects.

Variable	Neurological Controls (n=20)	OMS Subgroups				All OMS (n=54)
		Untreated (n=11)	ACTH (n=23)	Steroids (n=11)	Other (n=9)	
age at testing (years)	5.9±0.8	4.3±0.8	4.1±0.7	5.5±1.6	3.5±0.5	4.3±0.47
gender (n)						
– male	9 (45%)	4	9	5	3	21 (39%)
– female	11 (55%)	7	14	6	6	33 (61%)
OMS etiology (n)						
– no tumor found		6	14	4	5	29 (54%)
– tumor found		5	9	7	4	25 (46%)
OMS onset (years)		1.8±0.2	1.6±0.2	1.9±0.3	1.8±0.4	1.7±0.1
OMS duration (years)		2.5±0.8	2.4±0.7	3.6±1.5	1.7±0.5	2.6±0.5
OMS severity category (n)						
– mild		6	14	6	4	30 (56%)
– moderate		3	7	4	4	18 (33%)
– severe		2	2	1	1	6 (11%)
OMS duration category (n)						
– acute		2	3	3	1	9 (17%)
– subacute		3	7	3	2	15 (28%)
– chronic		6	13	5	6	30 (55%)
treatment duration (years)		–	1.9±0.7	2.6±1.3	0.58±0.18	1.8±0.5
previously treated (n)		7	14	10	4	35 (65%)

Data are means±SEM. There were no statistically significant differences between subgroups

clinically efficacious in OMS, inducing a response in a few to several days, so differential effects of ACTH and steroids would be of interest. Both agents could exert direct effects on neurotransmission [23] as well as indirect effects through their immunological disease-modifying properties, so non-steroidogenic immunotherapy was included as another treatment control group.

Patients and Methods

Subjects

Fifty-four children with OMS were recruited through the National Pediatric Myoclonus Center, examined by the same child neurologist (M.R.P.), and their parents signed consent for this Institutional Review Board-approved study. A thorough search had been made for occult neuroblastoma using neuroimaging and blood and urine tumor markers, and those with a tumor had undergone tumor resection.

Children were enrolled in this cross-sectional study at any time during their clinical course. Lumbar puncture was done as a part of routine immunological evaluation, and extra CSF was taken for this study. Some were actively being treated with ACTH, steroids, or non-steroid immunotherapy. ACTH-treated children were receiving intramuscular injections [H.P. Acthar® gel, 80 IU/mL, Questcor, Union City, CA], usually begun at a high dose (75 IU/m^2 twice a day, daily, then alternate days) and gradually tapered over several months or years [27]. Corticosteroid-treated children typically were taking oral prednisone or prednisolone, started at 2 mg/kg and then tapered, based on clinical response.

The majority of subjects had been treated previously (**Table 1**). However, only 2 of the ACTH group had received steroids, and 2 of the steroids group had received ACTH. A group designated as 'Untreated' had no prior therapy or had been treated with intravenous immunoglobulins (with or without other immunotherapy), which were discontinued 1.4±0.7 years (range: 0.04–5) prior to testing. The group 'Other' denotes non-steroidogenic types of immunotherapy, such as intravenous immunoglobulins (n=4), azathioprine (n=2), mycophenolate mofetil (n=1), and cyclophosphamide (n=2).

Neurological controls were 20 immunotherapy-naïve children, whose lumbar puncture was part of a diagnostic evaluation for heterogeneous neurological disorders: ataxia (n=1), autism (n=4), headache (n=1), developmental delay (n=4), epilepsy (n=5), myoclonus (n=3), other neurological disorders (n=2). They were age-matched with the OMS group.

Scoring of neurological status

To establish severity categories, each child with OMS was videotaped using a standardized format. A trained observer (E.D.T.), who was blinded to treatment status, rated motor impairment using the OMS Evaluation Scale, which we devised and validated [28]. Each item of the 12-item scale was rated from 0 to 3 as an index of increasing neurological severity or impairment. The total score was calculated as the sum of subscores, a score of 36 indicating maximum abnormality.

Lumbar puncture

Atraumatic lumbar puncture can be a challenge in the pediatric population, but especially so in young children with OMS, who have behavioral problems and paradoxical reactions to sedatives, which also provide them with inadequate sedation [40]. As a result, we do the procedure under anesthesia. It was performed in the left lateral decubitus position under intravenous propofol anesthesia after brief sevoflurane mask induction [40]. Propofol [2, 6-diisopropylphenol], the i.v. drug of choice for outpatient anesthesia, results in rapid loss of consciousness, fast recovery with less residual sedation and nausea or vomiting than with other sedative-hypnotics, and does not require intubation [4, 35]. This approach helps prevent contamination of CSF with blood due to trauma, which otherwise can create artifacts [15], standardize the degree of stress on brain neurochemistry, and provide compassionate care. Lumbar punctures were performed at approximately 10–11 a.m. To control for effects of diet on CSF

amino acids, all children were fasted overnight. To control for possible CSF gradient effects [2], the first 3 mL were sent for routine studies and an additional 1 mL was collected for amino acid analysis. CSF samples were stored at −80 °C.

Amino acid analyzer

Amino acid analysis was performed (M.C., B.T.) utilizing a commercial amino acid analyzer (Beckmann System 6300 High Performance Amino Acid Analyzer) with post-column derivatization with ninhydrin [16] to detect alanine, arginine, aspartic acid, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, serine, taurine, theonine, tyrosine, and valine. At the pH of the system, glutamate is glutamic acid and aspartate is aspartic acid. Although the analyzer is also capable of detecting asparagine, carnosine, citrulline, cystathionine, cystine, ethanolamine, homocystine, hydroxyproline, proline, sarcosine, tryptophan, and other compounds in different body fluids, those amino compounds are not detectable in CSF using this method.

Briefly, 100 μL of frozen CSF and 100 μL of internal standard [*S*-2-aminoethyl-L-cysteine (AEC) at 1 nmol/μL concentration] were added to a 1.5-mL microcentrifuge tube followed by addition of 20 μL of a 35 % (w/v) solution of sulfasalicylic acid (SSA) solution to precipitate polypeptides present in the CSF specimen. After thorough mixing on a vortex mixer, samples were centrifuged in an Eppendorf microcentrifuge Model 5415 at 13 000 rpm for 4 min. The supernatant was then filtered through a 0.2 μm filter and loaded into the metering loop of a Beckmann autosampler sample coil. 50 μL of sample were then injected by the autosampler onto a 10 cm × 4 mm high performance liquid chromatographic ion-exchange column packed with 5 μm beads. Individual amino acids were eluted from the column by gradient elution chromatography using commercially available buffers supplied by Beckmann. After emerging from the column, the effluent was mixed with a commercially available ninhydrin solution (Beckmann) maintained at 60 °C and individual amino acids were detected as their ninhydrin derivatives by dual-wavelength absorption spectroscopy at 440 and 570 nm.

The integrated detector response (peak area) was determined from the output of the chromatograph detector utilizing the System Gold chromatographic software (Beckmann) with appropriate baseline correction and peak separation parameters. Individual amino acids were then quantified by comparing the integrated detector response at 440 nm (proline, hydroxyproline) or 570 nm (all other amino acids) with the integrated detector response for the AEC internal standard after correction for differences in detector response factors for each individual amino acid. The response factors were determined by injecting known quantities of each amino acid along with the internal standard and measuring the integrated detector response to establish the ratio of nmoles amino acid/peak area (arbitrary units). Appropriate calibrators and controls were included in each batch analysis.

Published reference ranges for CSF amino acids in children are few [22]. There was a study of 50 healthy children, aged 3–18 years, in which CSF amino acids were analyzed by an amino acid analyzer using ion-exchange chromatography with fluorimetric detection [6]. Those data compare favorably with our own data on anesthesized neurological controls.

Gas chromatography-mass spectrometry

GABA was analyzed (J.R. C.) by GC/MS after purification from CSF with modifications previously described [5,32]. For sample purification, CSF (100 μL) was allowed to warm to room temperature and 10 μL (100 pmoles) GABA-d_6 were added. The samples were diluted in 500 μL 1 mM HCl and applied to a strong cation exchange column. The resin used for the study was AG 50W-X8, 100–200 mesh and hydrogen form (Bio-Rad Laboratories, Hercules, CA). The column was washed with 1.5 mL 1 mM HCl. GABA was eluted with 1.5 mL 6 N NH_4OH and was taken to dryness in a speed vac.

For derivatization, the samples were esterified by heating with 1 N HCl in propanol for 1 h at 70 °C. The reagent was evaporated in a speed vac and 100 μL heptafluorobutyric anhydride/EtOAc (1:3) were added and the samples heated for 15 min at 70 °C. Derivatized samples were analyzed on a Varian 3400 gas chromatograph interfaced to a Finnigan SSQ 7000 mass spectrometer (San Jose, CA) operated in the electron ionization mode. The GC column used for the study was a DB-1 (15 m, 0.2 mm i.d., 0.33 μm film coating, P.J. Cobert, St. Louis, MO). A linear temperature gradient was used. The initial temperature of 100 °C was held for 5 min and increased to 270 °C at 20 °/min. The temperature was held at 270 °C for 1 min. The source temperature, electron energy and emission current were 200 °C, 100 eV, and 300 μA, respectively. The injector and transfer line temperatures were 250 °C. The ions used for detecting analyte and internal standard were m/z = 282, 288, 254 and 260.

Statistical analysis

Statistical analyses were performed on Microsoft Excel and SPSS. One-way analysis of variance (ANOVA) was used to make comparisons between controls and one OMS category at a time (to provide sufficient statistical power), such as severity (mild, moderate, severe), duration (acute, intermediate, chronic), treatment status (currently untreated, ACTH-treated, steroid-treated, other immunotherapy), or ACTH dose (low dose, high dose). Post-hoc comparison of the means was made by the Duncan test. Pearson correlations were used to test for a relation between total score and amino acid concentration and a correlation between amino acids, but the more stringent $p < 0.005$ was used to obviate the effect of multiple comparisons. A chi-square (χ^2) test of independence was done to see if frequency counts between the four treatment groups differed.

As a first approach, statistical analysis was done on the entire dataset to probe the relationship between effects of three independent variables (treatment, OMS duration, and OMS severity) on CSF amino compound concentration. Treatment had a significant effect only on alanine ($F = 4.3$, $p = 0.013$), glutamine ($F = 3.3$, $p = 0.035$), lysine ($F = 4.8$, $p = 0.008$), phenylalanine ($F = 3.7$, $p = 0.022$), and taurine ($F = 6.5$, $p = 0.002$). To evaluate the possibility that treatment effects were confounded by interactions with OMS severity, duration, or both, between-subjects factors were further analyzed. There were no statistically significant interactions.

Results

There was no statistically significant difference between treatment subgroups in patient age at evaluation, gender, age at OMS onset, or in OMS duration, etiology, or severity. CSF leukocyte counts, protein and glucose were normal.

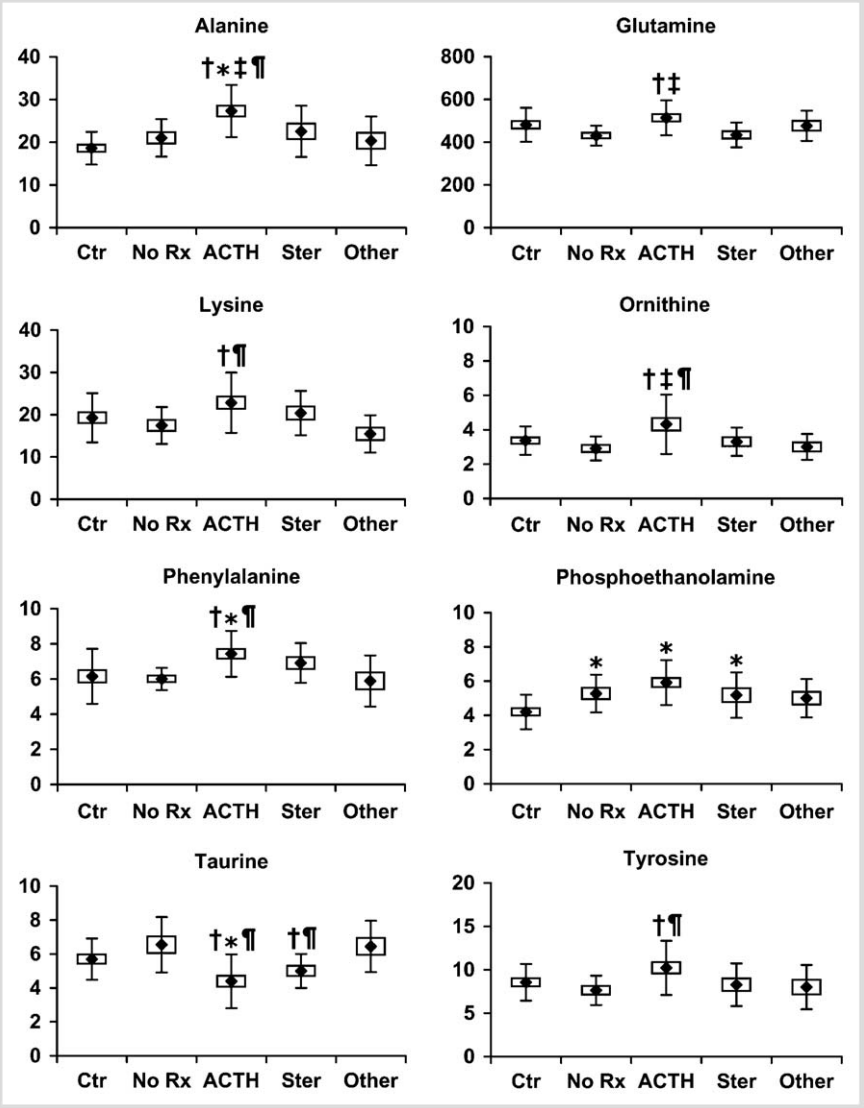

Fig. 1 CSF amino acid concentrations in controls (Ctr) and four OMS subgroups: untreated (No Rx), ACTH-treated (ACTH), steroid-treated (Ster), and non-steroidogenic immunotherapy (Other). Only those amino acids for which there were significant results are shown. The box-and-whisker graph indicates the mean (dot), SEM (box), and SD (whisker). Statistically significant main effects (ANOVA) were found for alanine ($F = 7.3$, $p = 0.000060$), glutamine ($F = 3.7$, $p = 0.0083$), lysine ($F = 3.2$, $p = 0.019$), ornithine ($F = 3.8$, $p = 0.0083$), phenylalanine ($F = 4.2$, $p = 0.0040$), phosphoethanolamine ($F = 4.3$, $p = 0.0038$), taurine ($F = 6.8$, $p = 0.00011$), and tyrosine ($F = 2.7$, $p = 0.037$). Post-hoc statistical comparisons between each OMS subgroup and controls (*), untreated OMS (†), steroids (‡), and other immunotherapies (¶) were made by the Duncan test, $p < 0.05$. Published means ± SEM (μmol/L) from healthy children include alanine 20.3 ± 0.66, glutamine 496 ± 11.5, ornithine 3.95 ± 0.14, phenylalanine 8.18 ± 0.54, phosphoethanolamine 4.87 ± 0.15, taurine 6.15 ± 0.17, and tyrosine 7.99 ± 0.26 (ref [6]).

Untreated OMS vs. neurological controls

In the children with OMS who were not on immunotherapy at the time of testing (**Fig. 1**), the only amino compound concentration that was abnormal was phosphoethanolamine, which was higher in OMS than in controls (+20%). There were no other statistically significant differences in amino acid levels, including GABA (controls 1.53 ± 0.13, OMS 1.69 ± 0.12 μmol/L), glycine (controls 5.00 ± 0.51, OMS 4.82 ± 0.26 μmol/L), and aspartic acid (controls 2.85 ± 0.22, OMS 2.36 ± 0.28). Glutamic acid was found sporadically and at too low a concentration for a mean to be reliably calculated. It was detected in 26 of 45 children with OMS (58%), but only 5 of 21 controls (24%), possibly indicating a higher frequency of detection in OMS ($p = 0.010$, chi-square). About half of the amino compounds were detected in controls at concentrations below 10 μmol/L.

Effect of treatment

In ACTH-treated children (**Fig. 1**), the concentrations of alanine (+51%), phenylalanine (+24%), and phosphoethanolamine (+44%) were significantly higher compared to neurological controls; the taurine level was 26% lower. Compared to untreated OMS, ACTH treatment was associated with higher concentrations of alanine (+30%), glutamine (+19%), lysine (+30%), ornithine (+48%), phenylalanine (+23%), and tyrosine (+34%); the taurine level was 32% lower. ACTH did not alter CSF GABA (1.37 ± 0.18 μmol/L), glycine (4.74 ± 0.18 μmol/L), or aspartic acid (2.32 ± 0.25).

To determine if ACTH effects merely reflected differences in OMS severity or duration, we looked for frequency differences between subgroups. In ACTH-treated OMS, the proportion of children in mild, moderate, and severe categories was not significantly different from other OMS subgroups. There were also no significant frequency differences in duration categories.

To look for a possible ACTH dose effect (**Fig. 2**), the ACTH data were divided clinically into two groups: ACTH 40 IU/m^2/day as "high dose" ($n = 5$), and <40 IU/m^2/day as "low dose" ($n = 18$). An ACTH dose effect was most apparent for alanine and phosphoethanolamine, for which both the higher and lower doses differed stepwise from controls and each other. The high dose group exhibited greater concentrations for alanine (+29%), arginine (+20%), lysine (+65%), ornithine (+50%), and phosphoethanolamine (+30%), compared to the low ACTH dose. There were also some statistically significant dose-associated differences for amino compounds that were not significant in the overall ACTH group.

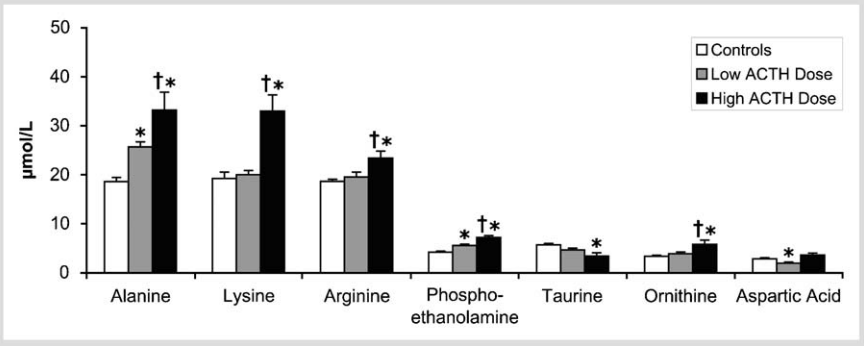

Fig. 2 Effect of ACTH dose on CSF-amino compound concentration. Data are means ± SEM. ANOVA revealed statistically significant main effects for alanine ($F = 23.6$, $p = 1.7 \times 10^{-7}$), lysine ($F = 14.6$, $p = 0.000017$), phosphoethanolamine ($F = 18.1$, $p = 2.6 \times 10^{-6}$), ornithine ($F = 7.3$, $p = 0.0012$), aspartic acid ($F = 7.2$, $p = 0.0021$), arginine ($F = 4.3$, $p = 0.020$), and taurine ($F = 6.4$, $p = 0.0040$). Post-hoc statistical comparisons between ACTH groups and controls (*) and high vs. low ACTH dose (†) were made by the Duncan test, $p < 0.05$.

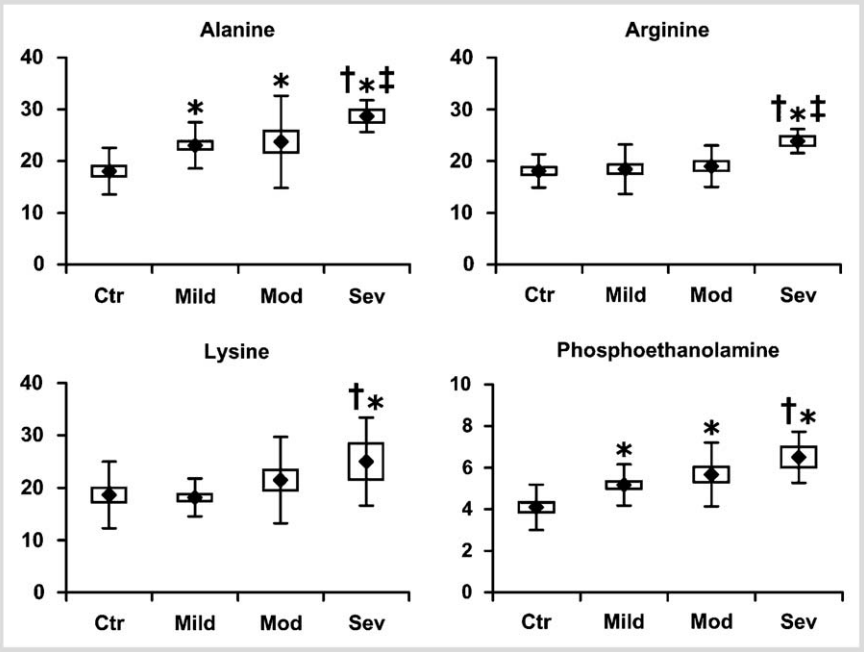

Fig. 3 Effect of OMS motor severity on CSF amino acid concentrations. Only those amino acids for which there were significant results are shown. Severity categories were based on total score as follows: mild if 0–12 (n = 30), moderate (Mod) if 13–24 (n = 18), severe (Sev) if 25–36 (n = 6). ANOVA revealed statistically significant main effects for alanine ($F = 5.9$, $p = 0.0012$), arginine ($F = 3.4$, $p = 0.022$), lysine ($F = 2.8$, $p = 0.048$), and phosphoethanolamine ($F = 8.3$, $p = 0.000084$). Post-hoc statistical comparisons between each severity category and controls (*), severe vs. mild (†), and severe vs. moderate (‡) were made by the Duncan test, $p < 0.05$.

Corticosteroid treatment had no significant effect on CSF amino compounds, except for 24% lower taurine compared to untreated OMS but not to controls. CSF amino compounds were not different from controls in the corticosteroid-treated group, except for the higher phosphoethanolamine level found in other OMS groups. Corticosteroid treatment was associated with a lower concentration of alanine, glutamine, and ornithine compared to ACTH treatment.

The non-steroidogenic immunotherapy group did not differ significantly from neurological controls or untreated OMS.

Effect of OMS severity

When all OMS subgroups were combined, OMS severity (**Fig. 3**) was associated with a higher concentration of four amino acids: alanine, arginine, lysine, and phosphoethanolamine. The levels were normal or slightly higher in mild OMS and increased with neurological severity to above control levels. Severe OMS had the highest levels of alanine (+59%), arginine (+31%), lysine (+34%), and phosphoethanolamine (+59%) compared to controls. No significant effect of OMS severity was found for other amino compounds.

When OMS severity category was evaluated without the ACTH group (data not shown), effects of OMS on both alanine ($F = 6.4$, $p = 0.0010$) and phosphoethanolamine ($F = 4.7$, $p = 0.0059$) remained highly significant and exhibited the same pattern; those on arginine and lysine did not.

In the ACTH-treated group, OMS severity (total score) was highly correlated with lysine ($r = 0.60$, $p = 0.0026$) and valine ($r = 0.56$, $p = 0.0059$). There were no significant correlations in the currently untreated OMS group.

Effect of OMS duration

The duration of OMS (**Fig. 4**) also had a significant effect on several, but not all amino acids (ANOVA). Three patterns were found. For alanine and phosphoethanolamine, the concentration was elevated in the acute or subacute subgroup (+44% for alanine, +56% for phosphoethanolamine) and declined toward control values with increasing OMS chronicity. For aspartic acid, the concentration also declined with increasing OMS chronicity (−28% in the chronic group) but was at control levels in the acute subgroup. For methionine, the concentration was low in the acute group, but not the other groups.

When OMS duration category was analyzed without the ACTH group (data not shown), the effects of OMS on alanine ($F = 3.1$, $p = 0.035$) and phosphoethanolamine ($F = 7.2$, $p = 0.00047$) remained significant and exhibited the same pattern; those on aspartic acid and methionine did not.

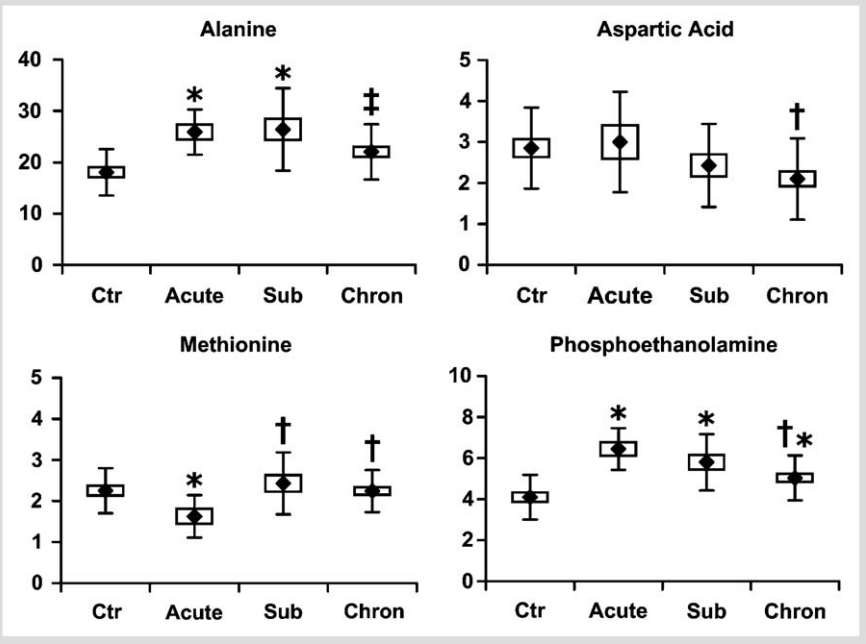

Fig. 4 Effect of OMS duration on CSF amino acid concentrations. Only those amino acids for which there were significant results are shown. Duration categories were assigned as follows: acute if ≤0.3 year (n = 9), subacute (Sub) if >0.3 ≤ 1 year (n = 16), chronic (Chron) if >1 year (n = 29). ANOVA revealed statistically significant main effects for alanine ($F = 6.9$, $p = 0.00039$), phosphoethanolamine ($F = 10.5$, $p = 0.0000089$), aspartic acid ($F = 3.0$, $p = 0.036$), and methionine ($F = 3.8$, $p = 0.013$). Post-hoc statistical comparisons between each OMS duration category and controls (*), acute vs. subacute or chronic (†), subacute vs. chronic (‡) were made by the Duncan test, $p < 0.05$.

Interrelation of CSF amino acids

There were significant correlations between amino acids at the $p \leq 0.005$. level. The correlations differed among controls (data not shown) and the various OMS subgroups. Only leucine and isoleucine were correlated consistently in each of the groups.

Discussion

ACTH therapy in OMS was associated with altered concentrations of several different CSF amino compounds which, for the most part, were not mimicked by corticosteroids. There was also a significant relationship of CSF phosphoethanolamine and alanine to OMS severity and duration, but the number of subjects in each subgroup was small. No effects of therapy or OMS on GABA or glycine, the amino acids more commonly associated with human myoclonic disorders, were found. The data from this study should be considered in the context that CSF levels of amino acids, even ones not usually considered clinically, are alterable by disease and by certain drugs. Multiplicity rather than selectivity of effects seems to epitomize CSF amino compound studies in most other neurological disorders, such as epilepsy [3,4,18,30], infantile asphyxia [8], late onset ataxias [12], depression [17], migraine [32], meningitis [30,34], myelopathy [38], Parkinson's disease [19], and multiple sclerosis [21]. We are unaware of any previous CSF amino compound studies in paraneoplastic disorders.

As to the relation between the various amino compound perturbations, there was no simple structural commonality based on the number of amino or carboxylic groups, carbon chain length or configuration, net charge (neutral, basic, acidic), molecular size, side chain or (R) group substitution. The metabolic fate of the amino acids (glycogenic, ketogenic, or both) also did not provide an explanation. Two of the aromatic amino acids involved in catecholamine metabolism were increased. Although tryptophan was undetectable by the amino acid analyzer, we have shown previously that its CSF concentration is normal in OMS [10].

Do these data suggest ACTH effects on brain metabolism? Although the equilibrium between serum and cerebral amino acids is dynamic, the exchange of non-essential amino acids is much less than that of essential amino acids, children included [39]. Alanine, like other small neutral amino acids (GABA, glycine, proline), is synthesized by the brain, and access to the brain from the circulation is very restricted; such is the case for asparagine, isoleucine, phosphoethanolamine, phosphoserine, taurine, and valine [20]. Elevated alanine may reflect increased nitrogen removal from brain. Elevated phosphoethanolamine, a precursor for phosphatidylethanolamine, may indicate increased brain phospholipid turnover. Phospholipids are important for neural membrane synthesis and signal transduction [31]. Higher CSF concentrations of glutamine, the most abundant amino acid in CSF, may reflect an abnormality of the brain glial-neuronal glutamine/glutamate cycle, such as increased enzymatic detoxification of glutamate, or associated with *N*-methyl-D-aspartate receptors [43].

There was no abnormality of the major inhibitory amino acid neurotransmitters in OMS. The lack of change in free GABA is an important negative finding in view of the significant reduction in free GABA found in some myoclonic disorders, such as progressive myoclonus epilepsy [1], and other epilepsies, such as infantile spasms [18]. However, total GABA also consists of homocarnosine and L-pyrrolidinone, which were not measured. Propofol enhances the activity of the γ-aminobutyric acid (GABA)-activated chloride channel [9]. However, while our results may have been different in the absence of anesthesia, neurological controls and children with OMS both received the same anesthetic regimen.

The CSF amino acid profile was more altered by treatment with ACTH than with steroids. Because cross-sectional studies can be influenced by differences in drug dosage or dosing schedules, these findings need to be replicated in a time-course treatment study. The differential properties of ACTH and corticosteroids are of interest, because the clinical effect of ACTH is more rapid and complete in OMS [26]. Melanotropinergic neurons may mediate some of the pleotropic properties of ACTH in brain [41]. The only shared treatment effect was lower taurine. Taurine, which is accumulated to millimolar levels in the brain, appears to play an important role in several physiological processes, such

as neuromodulation, acting as a partial agonist at $GABA_A$ and glycine receptors [11].

The main limitation of this study is that larger subgroups would be necessary for secondary analysis or multivariate analysis. Also, interpretation of amino compounds found at low concentrations in CSF must be cautious. Finally, the absence of a CSF abnormality or change does not rule out a structure- or cell type-specific change that is not manifested in CSF.

In conclusion, ACTH altered CSF concentrations of the neurotransmitter precursors phenylalanine and tyrosine, the putative neurotransmitter taurine, and the glutamine metabolite glutamine. However, it had a unique pattern of effects on non-neurotransmitter amino compounds, such as alanine, that were dose-related. Because these neurochemical effects were not shared by corticosteroids, it is tempting to speculate that some may contribute to the differential clinical efficacy of ACTH and steroids. However, both have a multiplicity of neural and immunological effects, which we are continuing to investigate.

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