

Cerebrospinal Fluid ACTH and Cortisol in Opsoclonus-Myoclonus: Effect of Therapy

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Opsoclonus-myoclonus syndrome is one of a few corticotropin (ACTH)-responsive central nervous system disorders of childhood. We measured cerebrospinal fluid ACTH and cortisol in 69 children with opsoclonus-myoclonus and 25 age- and sex-matched control subjects to determine endogenous levels and look for hypothesized differential hormonal effects of ACTH and corticosteroid treatment. Cerebrospinal fluid cortisol was 10-fold higher with ACTH treatment (n = 26), but was unchanged with oral steroid treatment (n = 18) or no treatment (n = 25). It was significantly higher in children receiving daily high-dose ACTH than alternate day ACTH. In ACTH-treated children, cerebrospinal fluid and serum cortisol were highly correlated ($r = 0.96$, $P = 0.0001$), with a mean ratio of cerebrospinal fluid to serum cortisol of approximately 1:10. Cerebrospinal fluid ACTH concentration did not differ significantly between untreated opsoclonus-myoclonus and control subjects but was lower with ACTH (−29%) or steroid treatment (−36%), suggesting feedback inhibition of ACTH release. These data delineate differences in the central effects of ACTH and corticosteroid therapy, as well as between high and low ACTH doses, and support the integrity of the brain–adrenal axis in pediatric opsoclonus-myoclonus. © 2005 by Elsevier Inc. All rights reserved.

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Introduction

Adrenocorticotrophic hormone (ACTH) and corticosteroids are the two most commonly prescribed treatments for opsoclonus-myoclonus syndrome, an autoimmune-mediated neuropsychiatric disorder of childhood that can be associated with neuroblastoma [1]. ACTH was introduced by Kinsbourne in his original case reports on opsoclonus-myoclonus [2]. Since then, many others have found ACTH to be efficacious, but there are several styles of administration [3]. In 1990, the National Pediatric Myoclonus Center modified the 150 IU/m² high-dose ACTH protocol for infantile spasms [4] by introducing plateaus and lengthening the period of tapering, later demonstrating efficacy in opsoclonus-myoclonus [5]. After 2 weeks of daily dosing, the dosing schedule decreases to alternate days to reduce side effects. Since then, we extended the protocol to 40 weeks, because of the high incidence of relapse in opsoclonus-myoclonus [6]. A different style of practice is to administer ACTH daily for the entire duration of its use. Still others favor a low rather than high dose schedule.

In contrast, corticosteroid treatment, which usually involves oral prednisone or prednisolone or intravenous pulse doses of methylprednisolone [3], is preferred by some for ease of administration, concerns over ACTH-induced side effects, or unfamiliarity with ACTH therapy. In our experience, corticosteroids are much less efficacious at inducing a remission, especially in children with moderate or severe symptoms, but there have been no head-to-head clinical trials or mechanistic data to support the use of one over the other.

The mechanism of action of ACTH and corticosteroids in opsoclonus-myoclonus is unclear, because both are

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Table 1. Clinical information

Variable	Controls	Opsoclonus-Myoclonus			
		All	Untreated	ACTH	Steroids
Sample size (n)	25	69	25	26	18
Age (yr)	6.7 ± 1.1	4.4 ± 0.4	4.9 ± 0.6	4.2 ± 0.7	4.1 ± 1.0
Sex					
Male (n)	8 (32%)	35 (51%)	15 (60%)	10 (38%)	10 (56%)
Female (n)	17 (68%)	34 (34%)	10 (40%)	16 (61%)	8 (44%)
Etiology					
No tumor (n)	—	40 (58%)	19 (76%)	14 (54%)	7 (39%)
Tumor (n)	—	29 (42%)	6 (24%)	12 (46%)	11 (61%)
Onset (yr)	—	1.8 ± 0.1	1.8 ± 0.2	1.9 ± 0.2	1.7 ± 0.2
Duration (yr)	—	2.7 ± 0.4	3.1 ± 0.6	2.3 ± 0.7	2.5 ± 1.0

Data are numbers of subjects with percentages or means ± S.E.M. There were no statistically significant differences between subgroups.

capable of exerting central effects as well as immunosuppression [3,7]. Because ACTH potently stimulates release of glucocorticoids, most effects of ACTH (like cortisol) on the brain have been attributed to activation of glucocorticoid receptors. More recently, direct effects of ACTH on neurons via melanocortin receptors have been proposed [8], but the penetrance of ACTH across the blood–brain barrier in humans is unknown and estimated to be quite low [9,10]. ACTH and corticosteroid therapy may exert differential effects on brain ACTH and cortisol (hydrocortisone, compound F) concentrations, but posttreatment and endogenous levels of ACTH and cortisol in opsoclonus-myoclonus have not been determined previously.

This article reports on the measurement of ACTH and cortisol primarily in cerebrospinal fluid but also in some blood samples in a cross-sectional study of pediatric opsoclonus-myoclonus. We proposed that ACTH dose and dosing schedule would be important clinical variables, hypothesizing that higher doses and daily dosing were apt to exert more effect on cerebrospinal fluid ACTH and cortisol.

Materials and Methods

Study Group

Sixty-nine children with confirmed opsoclonus-myoclonus were recruited through the National Pediatric Myoclonus Center over a 5-year period to be enrolled in this cross-sectional study, which was approved by the Institutional Review Board, as a part of their comprehensive immunologic evaluation. Some were being treated with ACTH (Acthar gel) by intramuscular injection or with oral corticosteroids (prednisone, prednisolone, or dexamethasone), whereas others were untreated at the time of evaluation. Clinical (Table 1) and dose information were collected. Controls were age- and sex-matched children undergoing lumbar puncture as part of a diagnostic evaluation for pseudotumor cerebri, seizures, or movement disorders, including non-ACTH responsive myoclonic disorders, such as progressive myoclonus epilepsy.

Cerebrospinal Fluid Collection

Lumbar punctures were performed under propofol intravenous anesthesia to provide compassionate care, reduce stress that might alter hormone levels, and to decrease the likelihood of obtaining blood-

contaminated cerebrospinal fluid [11]. Propofol does not affect cortisol synthesis or the response to ACTH [12]. The procedure was performed mid-morning after overnight sleep and a minimum of 5-6 hours fasting, under sterile conditions in the left lateral decubitus position at L₄₋₅. Samples were placed on ice, aliquoted, and immediately frozen on dry ice for storage at -80°C. They were shipped on dry ice to Esoterix Endocrinology (Calabasas Hills, CA) for assays.

Cortisol Assay

Cerebrospinal fluid cortisol was measured at the Esoterix Inc. endocrinology laboratory with an in-house, highly sensitive cortisol radioimmunoassay procedure. Briefly, cerebrospinal fluid was centrifuged to remove particulate matter. 200 µL of cerebrospinal fluid samples in duplicate was extracted with hexane:ethyl acetate. Aliquots of the extract were evaporated to dryness in a rotary evaporator. The dried extract was redissolved in assay buffer, and the cortisol content was determined by radioimmunoassay analysis. Reaction mixture containing the sample, cortisol antibody, and the tracer (¹²⁵I-radiolabeled cortisol) was incubated and precipitated using 60% saturated ammonium sulfate. The assay mix was centrifuged, and supernatant was decanted. The bound fraction (pellet) was counted for radioactivity in a gamma scintillation counter. The amount of radioactivity measured is inversely proportional to the concentration of cortisol present. Each assay was set with a full set of cortisol reference standards spanning the analytical range of the assay. A standard curve was generated by plotting the response (radioactivity) versus the respective concentration of cortisol for each standard. Cortisol concentration in unknown and control samples was determined directly from the standard curve. The solvent extraction step permitted partial purification and concentration of samples before analysis in order to increase detection sensitivity [13,14]. The sensitivity of the method is 0.05 µg/dL using 200 µL of sample. The ultrasensitive cortisol assay had an inter-assay imprecision (coefficient of variation [CV]) of 14%, 12%, and 7% for target concentrations at 0.067, 0.20, and 0.54 µg/dL respectively.

For serum cortisol measurements, 50-µL serum samples were diluted 20-fold in cortisol buffer. Aliquots of the diluted sample were assayed for cortisol content by radioimmunoassay analysis as described above but used higher concentrations of standards corresponding to higher analytical range of the assay. The sensitivity of the serum cortisol method is 1.0 µg/dL based on a 50-µL sample. The serum cortisol assay had an inter-assay imprecision (CV) of 17%, 8.3%, and 7.7% for target concentrations at 1.6, 8.8, and 26.0 µg/dL respectively.

ACTH Assay

Cerebrospinal fluid ACTH concentration was measured at the Esoterix Inc. endocrinology laboratory using the Nichols (San Juan Capistrano,

CA) ACTH chemiluminescence immunometric assay [15,16]. This assay used paired monoclonal and polyclonal antibodies that bind specifically to defined amino acid regions of the ACTH molecule. The monoclonal antibody labeled with an acridinium ester binds to N-terminal region of ACTH, while the polyclonal antibody coupled to biotin binds to C-terminal region of ACTH. The addition of avidin-coated beads to the reaction mix allowed formation of an ACTH “sandwich” complex: avidin-coated bead/biotinylated ACTH antibody/ACTH/acridinium ester-labeled ACTH antibody. At the end of assay incubation, the beads were washed to remove unbound components. Washed beads were placed in the luminometer, which injects solution that oxidizes the acridinium ester. The emission of light generated in the reaction was quantified and expressed in relative light units by the luminometer. The amount of bound labeled antibody was directly proportional to the concentration of ACTH in the sample. A standard curve was generated in each assay by plotting the relative light units against the respective concentration of ACTH standards. The concentration of ACTH in the unknown sample was determined directly from the standard curve. The sensitivity of the assay is 0.5 pg/mL. The ACTH assay had an inter-assay imprecision (CV) of 6.9%, 5.6% and 5.6% for target concentrations at 11, 88, and 394 pg/mL respectively.

Plasma ACTH concentration was measured at Specialty Laboratories Inc. (Santa Monica, CA) also using the Nichols ACTH chemiluminescence immunometric assay. The assay sensitivity was 1 pg/mL.

Data Analysis

Data were analyzed statistically using one-way analysis of variance and two-tailed *t* tests on Microsoft Excel XP. They were graphed using the same software program and finalized in Adobe Photoshop 7.0. The Duncan test was employed as a post hoc multiple comparisons test on SPSS software. For correlational analysis, Pearson correlations were obtained. For all analyses, the level of statistical significance was set at $P < 0.05$.

Dose and dosing schedule were identified as two important independent variables for statistical analysis. To compare high-dose versus low-dose, the ACTH-treated group was divided at the median (32 IU/m²/day) and the steroid group was also divided at the median (1.5 mg/kg/day). In cases of alternate day dosing, the dose was halved as an approximation for comparison with the daily dose group. For analysis of the steroid-treated group, prednisone and prednisolone are approximately equivalent for dosing, but dexamethasone is more potent (0.75 mg = 5 mg prednisone), so the dose was converted accordingly. The biologic half-life for prednisone and prednisolone is 18-36 hours, but it is 36-54 hours for dexamethasone. In cases of alternate day or less frequent dosing, a daily dose was calculated by dividing the total dose by the number of days between injections.

Results

There were no statistically significant differences between groups in age, sex, etiology, onset or duration of opsoclonus-myoclonus, allowing tight comparisons to be made. ACTH and cortisol levels were detectable in all children. Patient age at testing was not significantly correlated either with ACTH or cortisol concentration. Cerebrospinal fluid ACTH and cortisol concentrations were not correlated (data not shown).

When control, untreated opsoclonus-myoclonus, and ACTH- or steroid-treated groups were compared (analysis of variance), there were significant group main effects on the cerebrospinal fluid concentration of ACTH ($F = 5.2$, $P = 0.002$) and cortisol ($F = 5.0$, $P = 0.003$). Neither ACTH nor cortisol concentrations were significantly dif-

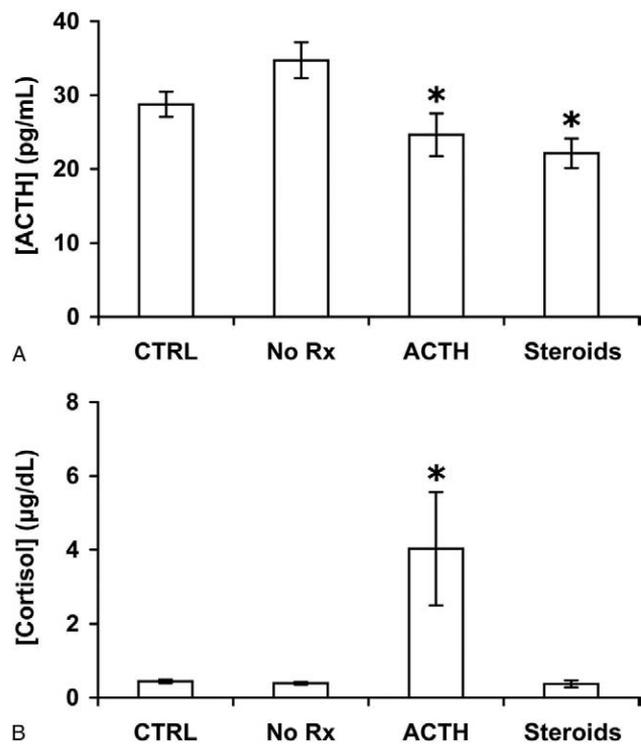


Figure 1. Cerebrospinal fluid ACTH (A) and cortisol (B) concentrations in neurologic pediatric control subjects (CTRL) ($n = 25$) and opsoclonus-myoclonus patients treated with ACTH ($n = 26$), steroids ($n = 18$), or no treatment (No Rx) ($n = 25$). After significant group main effects were demonstrated by analysis of variance, post hoc statistical comparisons were made by the Duncan test. For ACTH concentration, the ACTH- and steroid-treated groups differed from untreated opsoclonus-myoclonus or controls (which did not differ from each other). For cortisol concentration, the ACTH-treated group differed from all other groups (which did not differ from each other). Asterisk indicates $P < 0.05$.

ferent in untreated opsoclonus-myoclonus and control groups (Fig 1). However, the cerebrospinal fluid ACTH concentration was significantly lower in ACTH-treated (–29%) and steroid-treated (–36%) opsoclonus-myoclonus compared with untreated opsoclonus-myoclonus ($P < 0.05$, Duncan test). The level of cerebrospinal fluid cortisol was significantly increased by 10-fold in ACTH-treated children only ($P < 0.05$, Duncan test).

When alternate day ACTH dosing was compared with daily dosing (Fig 2), there was no significant difference in cerebrospinal fluid ACTH concentration. However, the cortisol concentration was 25-fold higher with daily ACTH dosing; the alternate day dosing group did not differ significantly from the control group. In corticosteroid-treated children, there was no significant effect of dose or dose schedule (Table 2).

In the ACTH-treated group (Fig 3), the concentrations of cerebrospinal fluid and serum cortisol were highly correlated ($P = 0.0001$). The mean ratio of cerebrospinal fluid to serum cortisol was approximately 1:10. Although ACTH dose and dosing schedule resulted in large differences in serum cortisol levels, the differences were not significant owing to markedly unequal variance. The mean for the daily ACTH treatment subgroup (68.5 ± 26.6

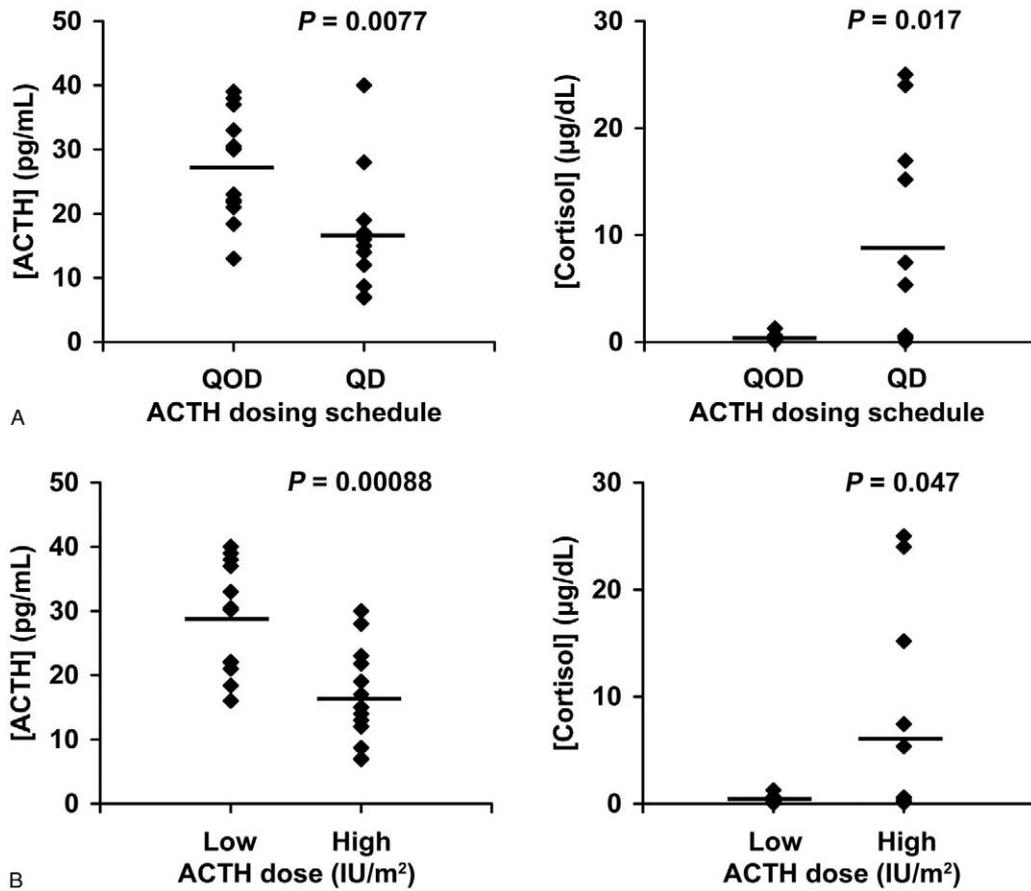


Figure 2. Effect of ACTH dosing schedule (A) and dose (B) on cerebrospinal fluid ACTH (left) and cortisol (right) concentrations. For dose analysis, the data were divided at the median ($32 \text{ IU/m}^2/\text{day}$) into "high" ($n = 13$) and "low" dose ($n = 12$), and alternate day dosing ($n = 14$) and daily dosing ($n = 11$). The concentration of ACTH was significantly lower in children receiving daily dosing (QD) or higher ACTH doses (High) compared with alternate day dosing (QOD) or lower ACTH doses (Low), *t* test. The concentration of cerebrospinal fluid cortisol was significantly higher in children on daily dosing or higher ACTH doses.

$\mu\text{g/dL}$) was 5.9-fold higher than the alternate day ACTH treatment subgroup (11.6 ± 2.9) ($P = 0.065$). The mean for the high-dose ACTH subgroup ($62.9 \pm 24.4 \mu\text{g/dL}$) was 3-fold higher than the low-dose ACTH subgroup (11.5 ± 3.3) ($P = 0.79$).

To determine if a postinjection peak in cerebrospinal ACTH concentration could have been missed by the study design, two children on alternate day low-dose ACTH

received ACTH injections 30-35 minutes before cerebrospinal fluid collection (Table 3). This time point was estimated to allow for transport of ACTH into the brain. Although serum cortisol and blood ACTH levels were increased, cerebrospinal fluid concentrations were not.

Table 2. Effect of corticosteroid dose and dose schedule on cerebrospinal fluid ACTH and cortisol concentrations

Variable	n	ACTH	Cortisol
Dose (mg/kg/day)			
"Low"	7	25.7 ± 4.0	0.28 ± 0.11
"High"	7	18.0 ± 2.2	0.39 ± 0.20
Dose schedule			
Alternate day	4	28.3 ± 6.5	0.40 ± 0.17
Daily	11	19.5 ± 1.8	0.31 ± 0.13

Data are means \pm S.E.M. The designation of "low" vs "high" dose was made by splitting the group at the median (1.5 mg/kg/day). For the purpose of arriving at a total daily dose, alternate day dosing was converted to the equivalent daily dosing. There were no statistically significant differences between groups.

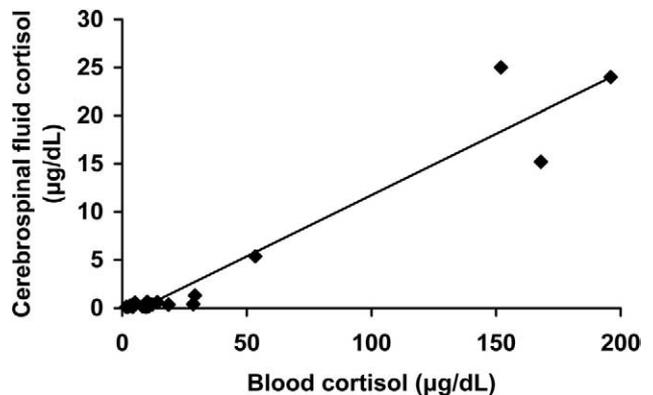


Figure 3. Relation of cerebrospinal fluid and serum cortisol in ACTH-treated children with opsoclonus-myoclonus. The Pearson correlation between the two ($r = 0.96$) was highly significant ($P = 0.0001$). The equation for linear regression was $y = 0.127x - 1.01$.

Table 3. Timed measurement of cerebrospinal fluid and serum ACTH and cortisol concentrations after ACTH injection

Patient	ACTH dose (IU/m ²)	ACTH (pg/mL)		Cortisol (μg/dL)	
		Cerebrospinal fluid	Plasma	Cerebrospinal fluid	Serum
1	35	27	1476	<1	28.5
2	53	16	—	<1	71.4
Controls (n = 25)	—	28.8 ± 1.7	—	0.44 ± 0.05	—
Reference range	—	—	5-46	—	8.7-22.4

Blood was drawn 20 minutes after the morning ACTH injection, and cerebrospinal fluid was obtained 30-35 minutes after injection. Both patients were on alternate day dosing. Recalculation as equivalent daily dosing would place them in the low-dose category.

Discussion

A novel finding of this study was that daily high-dose ACTH treatment dramatically raised the concentration of cerebrospinal fluid cortisol, but alternate day and low-dose ACTH did not. None of these dose and dose schedule effects has been reported before for cerebrospinal fluid, but for serum in infantile spasms, high-dose ACTH treatment causes a rapid and sustained increase in serum cortisol concentration, more than the pulse effect from oral steroids or lower doses of ACTH [17]. To the extent that cortisol is a factor in clinical response to ACTH therapy, these data support the use of high-dose ACTH protocols for opsoclonus-myoclonus as well as infantile spasms. In light of recent evidence of lymphocyte recruitment into the central nervous system in opsoclonus-myoclonus [18], it will be of interest to examine the effects of high-dose ACTH on the cerebrospinal fluid lymphocyte immunophenotype.

Elevated brain cortisol may contribute to the superiority of ACTH treatment over oral corticosteroids in inducing a neurologic remission in opsoclonus-myoclonus, but ACTH, like steroids, exerts so many neurotropic [7] and immunologic effects [3] that the relative contribution of brain cortisol remains uncertain. Prospective dose-response and time course studies would be useful. However, the capacity of glucocorticoids to help stabilize neuronal excitability, maintain neuronal integrity, and facilitate behavioral adaptation is well known [19-21].

There have been few previous studies in children of the effect of ACTH treatment on cerebrospinal fluid ACTH concentration. In cryptogenic infantile spasms, the cerebrospinal fluid ACTH concentrations declined in infants who responded rapidly to ACTH injections [22,23]. In the study of 16 infants by Heiskala [23], carboxymethylcellulose-prolonged ACTH was administered daily, tapered if response was rapid, and doubled if response was insufficient after 2 weeks (6-12 IU/kg). A 25% decline in the cerebrospinal fluid ACTH level occurred only in three rapid responders. Riikonen and Gupta [22] reported a slight, nonsignificant decline in 5 of 10 children with infantile spasms who were treated with 2-6 IU/kg of ACTH for 4-8 weeks. These nonsignificant trends were significant in our study of a larger sample size.

The reduction in cerebrospinal fluid ACTH induced by treatment with ACTH or corticosteroids suggests negative

feedback inhibition on the hypothalamic-pituitary level [24]. ACTH secretion is under the control of corticotrophin-releasing factor formed in the hypothalamus. Exogenous corticosteroids and endogenous cortisol stimulated by ACTH therapy reduce endogenous ACTH production by effects on cortisol-ACTH homeostatic regulation. Our data are consistent with these mechanisms.

We were not able to demonstrate an increase in cerebrospinal fluid ACTH concentration acutely following ACTH injection in two subjects, even though plasma ACTH was elevated. These data could be explained by negligible transport of ACTH into brain [9,10]. If, instead, the 30-minute postinjection sampling time was too long, any ACTH peak still would have been minimal to be gone by 30 minutes. Rigorous time course studies are necessary.

Cerebrospinal fluid and serum cortisol were highly correlated in the ACTH-treated group, but only a fraction of serum cortisol, the principal endogenous glucocorticoid in humans, gained entry into the cerebrospinal fluid (and presumably brain) in the present study. This suggests a blood-brain barrier for cortisol, which has not been demonstrated previously in pediatric patients. Several studies have found that unbound cortisol transport across the blood-brain barrier is mediated by multidrug resistance P-glycoprotein [25], and circulating cortisol cannot gain free access to brain even in humans [26]. Such a phenomenon would explain our data, but there may be other factors as well.

The data presented here do not support an abnormality of the brain-adrenal axis in the pathophysiology of opsoclonus-myoclonus or its responsiveness to ACTH treatment. The endogenous levels of ACTH and cortisol in cerebrospinal fluid were normal. In that respect, opsoclonus-myoclonus and infantile spasms, in which low concentrations of cerebrospinal fluid ACTH and cortisol are evident [27-30], are fundamentally different.

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