Cerebrospinal fluid GABA reductions in seizure patients evoked by cerebellar surface stimulation

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Lumbar cerebrospinal fluid (CSF) gamma-aminobutyric acid (GABA) levels determined by fluorometric assay in four seizure patients were found to be significantly lower during bilateral, continuous cerebellar stimulation than those determined after a 7-day period without stimulation. The CSF GABA concentrations during chronic unilateral, alternating cerebellar stimulation were reduced in three seizure patients but unchanged in a fourth patient. The percentage decrease in CSF GABA appeared to be independent of cerebellar stimulation frequency. These findings suggest that GABA-mediated neuronal transmission is depressed during cerebellar surface stimulation and this evoked reduction in GABA activity may compromise the efficacy of cerebellar stimulation in the treatment of epilepsy.

Lumbar CSF cyclic guanosine monophosphate levels determined by radioimmunoassay were not significantly altered by either mode or frequency of cerebellar stimulation.

KEY WORDS • gamma-aminobutyric acid (GABA) • cerebellar stimulation • epilepsy • cyclic guanosine monophosphate • cerebrospinal fluid

CHRONIC stimulation of the paleocerebellum and neocerebellum has been reported to decrease seizure activity in man.1-6 The mechanism for this anticonvulsant activity is unknown. Cerebellar surface stimulation was originally thought to activate Purkinje cells which in turn inhibit the cerebello-thalamo-cortical feedback loop.21 If this mechanism is dominant, then those epileptic patients with greater Purkinje cell densities should have more seizure suppression during cerebellar stimulation. However, our recent clinicopathologic report42 does not support this concept. In addition, our double-blind clinical trials do not confirm objective cerebellar stimulation-induced seizure inhibition.53

Gamma-aminobutyric acid (GABA) is the putative inhibitory neurotransmitter of Purkinje cells.6,13,19,20,35,37,50 The increase in GABA levels reported in fourth ventricular perfusates during high frequency (200/sec) cerebellar stimulation in cats suggests the release of GABA from Purkinje cell axons.38 If Purkinje cells are activated during cerebellar surface stimulation, then GABA concentrations in the cerebrospinal fluid (CSF) should increase. This preliminary com-
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communication evaluates induced CSF GABA alterations in four epileptic patients with respect to unilateral alternating, and bilateral continuous cerebellar stimulation. Evoked CSF cyclic guanosine 3',5'-monophosphate (cGMP) alterations were also evaluated in these patients since a facilitation of GABA inhibitory transmission is associated with a decrease in the concentration of cGMP in the rat cerebellum. The variation of these induced neurochemical alterations with respect to stimulation frequency was also assessed.

Clinical Materials and Methods

This neurochemical evaluation included two patients with generalized tonic-clonic seizures (Cases 2 and 4), one patient with both complex partial and generalized tonic-clonic seizures (Case 3), and one patient with atypical myoclonus and generalized tonic-clonic seizures (Case 1). A detailed discussion of our clinical evaluation of cerebellar stimulation for the treatment of intractable epilepsy shall be the subject of a separate communication. The patients were all men, with a mean age of 29 ± 3 years (SEM).

These four seizure patients underwent implantation of two Avery E333 electrode arrays* over the paravermian regions of the anterodorsal cerebellum. The electrodes were connected via subcutaneous wiring to two Avery I110 radiofrequency receivers* placed subcutaneously on the chest (Fig. 1). Postoperatively, Avery 901 loop antennae (Fig. 1) connected to an Avery S227 transmitter* were taped over the subcutaneous receiver implants. With transepidermal inductive coupling, the patients were stimulated with 3- to 12-mA capacitively-coupled monophasic pulses with exponential decay and 1-msec pulse duration alternating between electrode arrays every 8 minutes. Three patients (Cases 2, 3, and 4) were stimulated with 10 pulses/sec, while the patient with atypical myoclonic epilepsy (Case 1) received 200 pulses/sec.

Neurochemical studies were initiated after chronic cerebellar stimulation. Unilateral, alternating cerebellar stimulation was maintained 6 months before the initial lumbar puncture. Stimulation was discontinued for 7 days after which a second lumbar puncture was performed. Thereafter the patients underwent 5 to 7 days of unilateral alternating cerebellar stimulation followed by 16 hours of bilateral, continuous stimulation before the third lumbar puncture.

For comparison 11 seizure patients with generalized tonic-clonic, complex partial and/or focal motor seizures underwent lumbar punctures and served as a reference group for this study. The mean age of these patients without cerebellar electrodes was 31 ± 4 years and seven were men.

All patients were maintained on low monoamine diets and received only their routine anticonvulsant medications during the 2 weeks before CSF sampling. Patients were restricted to absolute bedrest with bedpan and all oral intake was avoided during the

*Cerebellar stimulation equipment made by Avery Laboratories, Inc., 145 Rome Street, Farmingdale, New York.
18 hours preceding lumbar puncture. All lumbar punctures were performed in standard fashion with the patient in the lateral decubitus position at 9 a.m.\(^{58,59}\)

During each lumbar puncture, a 4-ml aliquot of CSF was collected into a tube after 22 ml of CSF had drained from the spinal needle. This sampling technique was intended to promote CSF circulation from the ventricular outlets to the lumbar sac and to insure that the same CSF aliquot was obtained from each patient so that CSF GABA concentration gradients\(^{11}\) would not bias our study. After collection, CSF samples were immediately placed on ice and then into storage at \(-70^\circ\) C within 30 minutes.\(^{11}\) The GABA content of each 4-ml CSF sample was determined in duplicate using ion-exchange column chromatography and fluorescence detection based on the reaction of orthophthalaldehyde with primary amines\(^{14,57}\) and reported in pmole/ml.

Additional 2-ml CSF samples were collected in the same manner immediately after obtaining the 4-ml CSF samples described above. The cGMP concentration of these CSF samples was determined by radioimmunoassay\(^{56,47}\) and reported in pmole/ml. Blood samples drawn immediately after each lumbar puncture were analyzed for anti-convulsant drug content.

**Results**

After 6 months of unilateral alternating cerebellar stimulation, the mean CSF GABA level for Cases 1, 2, and 3 was 48 ± 14 pmole/ml (SEM) (Table 1). After 7 days without cerebellar stimulation, the mean CSF GABA concentration for these three patients was 106 ± 46 pmole/ml. The CSF GABA levels for Case 4 was 81 pmole/ml during unilateral alternating cerebellar stimulation, and 82 pmole/ml after 1 week without stimulation.

The mean CSF GABA concentration for all four patients was 100 ± 33 pmole/ml after 1 week without stimulation, and 60 ± 29 pmole/ml after 5 to 7 days of unilateral alternating cerebellar stimulation followed by 16 hours of bilateral, continuous cerebellar stimulation. This reduction in CSF GABA was statistically significant (p < 0.02, paired two-tailed Student t-test) (Fig. 2).

The mean CSF GABA level for the 11 seizure patients without cerebellar electrodes was 135 ± 11 pmole/ml. Unilateral, alternating cerebellar stimulation; SEM = standard error of the mean.

**TABLE 1**

*Cerebrospinal fluid GABA concentrations in relation to cerebellar stimulation (pmole/ml)*

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**Fig. 2.** Reductions of cerebrospinal fluid (CSF) GABA concentration during cerebellar stimulation. Mean CSF GABA decrease of zero represents mean CSF GABA concentration (“baseline”) in four patients after 1 week without cerebellar stimulation. *Asterisk* indicates significant reduction in mean CSF GABA level after 5 to 7 days of unilateral cerebellar stimulation followed by 12 hours of bilateral, continuous cerebellar stimulation (p < 0.02, paired two-tailed Student t-test). Graph represents mean-standard error.
was 135 ± 11 pmole/ml and was not significantly different from that determined in Cases 1–4 after 1 week without cerebellar stimulation.

The patient in Case 1 with myoclonic epilepsy underwent CSF GABA decreases of 15 and 31 pmole/ml during 200/sec unilateral alternating and the combination of unilateral alternating plus bilateral continuous cerebellar stimulation, respectively. This evoked CSF GABA response represented a mean decrease of 44%. The mean CSF GABA reductions in the three epileptic patients undergoing 10/sec unilateral alternating and the combination of unilateral alternating plus bilateral continuous cerebellar stimulation were 53 ± 36 and 45 ± 9 pmole/ml, respectively. The GABA response in these three stimulated patients represented a mean decrease of 42 ± 10%.

Mean CSF cGMP levels of 6.9 ± 2.2, 7.2 ± 2.6, and 7.0 ± 2.3 pmole/ml were determined after 6 months of unilateral alternating stimulation, 1 week without cerebellar stimulation, and the combination of 5 to 7 days of unilateral alternating cerebellar stimulation followed by 16 hours of bilateral continuous stimulation, respectively (Table 2). None of these alterations in mean CSF cGMP concentrations was statistically significant. The mean CSF cGMP concentration of 7.1 ± 2.8 pmole/ml determined in the 11 seizure patients without cerebellar electrodes was not significantly different from that noted in Cases 1–4 after 1 week without cerebellar stimulation.

Concentrations of anticonvulsant drugs in the blood remained in therapeutic range and did not vary significantly throughout the duration of our clinical investigation.

**Discussion**

Recent reviews imply that GABA may play a role in the neurochemistry of epilepsy. Experimental evidence suggests that GABA, an inhibitory neurotransmitter, causes both hyperpolarization and a large reduction in membrane resistance secondary to an increase in chloride ion permeability, which appears to provide a major defense against buildup and spread of epileptic activity. Both disrupted GABA metabolism and impairment of its action on the postsynaptic membranes have been shown to increase seizure susceptibility.

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<td>mean ± SEM</td>
<td>6.9 ± 2.2*</td>
<td>7.2 ± 2.6*</td>
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*No significant differences (paired two-tailed Student t-test) in mean cerebrospinal fluid (CSF) cyclic guanosine monophosphate (cGMP) concentrations determined after 6 months of unilateral alternating stimulation (Unilat. Stim.), 1 week without cerebellar stimulation (No Stim.), and 5 to 7 days of unilateral alternating cerebellar stimulation followed by 16 hours of bilateral continuous stimulation (Unilat. + Bilat. Cont. Stim.). Mean CSF cGMP level in reference group consisting of 11 seizure patients without cerebellar electrodes was 7.1 ± 2.8 pmole/ml. SEM = standard error of the mean.

Reductions in cerebral GABA concentrations in cats have been noted during cobalt-induced focal seizures. Levels of GABA were initially reported to be diffusely low in the cerebral cortex of seizure patients, however, later studies failed to demonstrate decreased GABA concentrations in epileptogenic foci excised from patients with focal cerebral cortical epilepsy. Recent preliminary reports indicate that CSF GABA levels of medicated seizure patients may not be significantly lower than those of unmedicated nonepileptic patients with other neurological disorders, but the CSF GABA concentrations in normal individuals have not yet been reported.

A recent literature review implies that chemicals that reduce animal brain GABA concentrations by approximately 40% produce spontaneous tonic-clonic seizures. Accordingly, the evoked mean reductions in CSF GABA of 37 ± 13% and 48 ± 9% in our patients who underwent long-term unilateral alternating and bilateral continuous cerebellar stimulation, respectively, were associated with mildly increased seizure activity. This finding supports reviews which imply no simple relationship between the onset of seizures and changes in GABA levels.
and suggests that the convulsive effect of these stimulation-evoked GABA reductions on epileptic activity may be compensated by other mechanisms. Specific cerebellar stimulation-induced elevations in CSF norepinephrine have been reported in our patients.\textsuperscript{58,59} This augmentation in noradrenergic activity raises seizure thresholds\textsuperscript{54} and may oppose the epileptogenic effects of these evoked reductions in GABA activity. These antagonistic neurochemical mechanisms may account for the variability of seizure activity noted during cerebellar stimulation.\textsuperscript{58,59}

When GABA is applied topically, injected vascularly or administered intraventricularly, it increases experimental seizure thresholds.\textsuperscript{17,43,52} Administered orally, GABA has been reported to suppress petit mal and generalized tonic-clonic seizures\textsuperscript{52} despite the inability of GABA to cross the normal blood-brain barrier.\textsuperscript{26}

Brain GABA levels may be elevated by inhibiting the mitochondrial catabolism of GABA by GABA-transaminase to succinic semialdehyde. Valproic acid (dipropylacetic acid) acts as a competitive inhibitor of GABA-transaminase and has been found to elevate whole-brain and cerebellar GABA content.\textsuperscript{25} These elevations in brain GABA levels correlate with the anticonvulsant effects of valproic acid against seizures in mice induced chemically,\textsuperscript{26} electrically,\textsuperscript{55} and audiogenically.\textsuperscript{44} In a recent review of 18 clinical series, 73\% of patients with generalized tonic-clonic seizures experienced better control during valproic acid therapy.\textsuperscript{45} If valproic acid exerts anticonvulsant activity through its relationship to brain GABA, then patients with depression of GABA metabolism during cerebellar stimulation may experience additional seizure suppression by taking valproic acid.

The source of the GABA in lumbar CSF cannot be determined from our study. In the cerebellum, basket-cell terminals on Purkinje cell bodies and endings of Purkinje cell axons on deep cerebellar nuclei\textsuperscript{27} or the lateral vestibular nucleus\textsuperscript{6} are GABA-mediated. In addition, small interneurons in the cerebral cortex,\textsuperscript{24} hippocampus,\textsuperscript{49} and thalamus\textsuperscript{19} release GABA. Levels of GABA in the spinal cord are low compared to those in the brain.\textsuperscript{12,16} The recent findings of low CSF GABA levels in patients with Huntington's disease\textsuperscript{15} associated with reduced brain GABA content\textsuperscript{46} and significant GABA concentration gradients in successive CSF aliquots obtained during lumbar puncture\textsuperscript{11} indicate that lumbar CSF GABA levels may reflect brain GABA metabolism.

The evoked CSF GABA reductions in our patients were probably cerebellar-mediated since manipulation of cerebellar stimulation was the only variable in our study. Fast frequency (200/sec) stimulation of the cat cerebellar cortex with depth electrodes produces Purkinje cell activation and GABA release into fourth ventricular perfusates; however, slow frequency (10 to 50/sec) stimulation produces no changes in perfusate GABA levels.\textsuperscript{56} Our data obtained using surface electrodes suggest that GABA-mediated neural transmission in man may be depressed by cerebellar surface stimulation, however this depression appears on preliminary study to be independent with relation to stimulation frequency. This conclusion is supported by more recent neurophysiological studies demonstrating reduced Purkinje cell discharge during cerebellar surface stimulation in cats\textsuperscript{6} and little effect on cortical evoked potentials in monkeys\textsuperscript{18} at low stimulation frequencies. These studies seem to confirm our clinicopathologic observations\textsuperscript{41} that the Purkinje cell does not appear to mediate the cerebellar stimulation-induced seizure suppression reported at slow stimulation frequencies.

The mean CSF GABA concentration in our patients (Cases 1–4) after 1 week without stimulation was similar to the mean steady-state level of the 11 seizure patients without cerebellar electrodes. These data suggest that stimulation-induced CSF GABA alterations return to approximately baseline levels within 1 week after the termination of cerebellar stimulation. The presence of cerebellar electrodes does not appear to contribute to the CSF GABA reductions observed during stimulation. In addition, the reported reduction in cerebellar GABA beneath the stimulating electrodes does not contribute significantly to the CSF GABA alterations noted in our study.\textsuperscript{51}

Iontophoretically applied cGMP increases the excitability of cerebral cortical neurons,\textsuperscript{48} and cerebellar levels of this nucleotide increase during experimentally-induced convulsions.\textsuperscript{27,28} An inverse relationship between
Gamma-aminobutyric acid levels in CSF

cGMP and GABA has been demonstrated in rat cerebellum following a variety of pharmacological manipulations.\textsuperscript{21}

Anticonvulsant drugs, phenytoin, phenobarbital, diazepam, and valproic acid, decrease cerebellar cGMP concentrations.\textsuperscript{26,28,29} Mutant mice deficient in Purkinje cells have reduced cerebellar cGMP levels, and these levels do not decrease further after administration of diazepam.\textsuperscript{30} Thus, cGMP appears to reside within Purkinje cells and may lower seizure thresholds.

Our study fails to demonstrate significant evoked cGMP alterations in lumbar CSF during cerebellar stimulation despite reductions in CSF GABA concentrations. Evoked cerebellar cGMP alterations may be intracellular, and thus not reflected in lumbar CSF, or may be masked by contributions from the spinal cord.\textsuperscript{29} Our patients have been chronically treated with anticonvulsant drugs that have been associated with Purkinje cell degeneration,\textsuperscript{2,9} therefore intracellular cGMP metabolism may fail to react to electrical stimulation.

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