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Elevated β-Endorphin in Cerebrospinal Fluid After Electrical Brain Stimulation: Artifact of Contrast Infusion?

Abstract. *β-Endorphin–like immunoreactivity in cerebrospinal fluid was assayed in 11 patients receiving electrical stimulation of the brain for chronic pain. Immunoreactivity increased dramatically after contrast ventriculography prior to stimulation. No further elevations were observed after stimulation. The magnitude and time course of elevations were identical after placement of electrodes either in the thalamus or in the periventricular gray matter. These results suggest that previous findings of stimulation-induced elevation of β-endorphin–like immunoreactivity in cerebrospinal fluid are attributable to an artifact of contrast ventriculography.*

Many studies have shown the effectiveness of electrical stimulation of the periventricular gray matter (PVG) (*1–6*) and various areas of the thalamus or internal capsule (*7–9*) for relief of chronic pain in humans. The most popular neurochemical hypothesis proposed to explain this phenomenon suggests that the effect of stimulation-produced analgesia (SPA) in humans is mediated by release of endogenous opiates. The hypothesis is based on evidence suggesting that (i) analgesia obtained through stimulation of the PVG is effective only in patients whose pain also responds to narcotics (*10*), (ii) intraventricular β-endorphin induces analgesia in patients (*11*), (iii) cross-tolerance occurs between morphine analgesia and SPA (*12, 13*), (iv) naloxone reverses SPA (*14*), and (v) SPA elevates levels of β-endorphin–like immunoreactivity (β-ELI) and methionine-enkephalin–like immunoreactivity in cerebrospinal fluid (CSF). However, several investigators have reported only slight or no reversal of SPA with naloxone administration (*8, 15*), and others have found either no change or inconsistent effects of PVG stimulation on CSF β-ELI (*5, 16*).

We have utilized electrical stimulation of the brain to relieve chronic pain in humans (*17*) and have examined the effects of such stimulation on CSF β-ELI. We report here our neurochemical results.

Four male and seven female patients underwent implantation of stimulating electrodes deep in the brain for a variety of algetic states (Table 1). All patients were completely free of opiate use at the time of the two-stage surgical procedure (*17*). Because some patients with pain of peripheral origin respond to thalamic stimulation as well as PVG stimulation (*9*) and because repeated PVG stimulation can lead to development of tolerance (*1*), all patients receiving a PVG electrode also received a thalamic electrode.

After insertion of a ventricular catheter into the frontal horn of the left lateral ventricle a 2-ml control sample of CSF was obtained. Metrizamide ventriculography was then performed to visualize the third ventricle and Sylvian aqueduct (*18*). In patients receiving a PVG electrode, target coordinates were 1 mm posterior and inferior to the posterior commissure and 1 mm lateral to the wall of the third ventricle. The target for the ventral posterior thalamic (VPT) electrode was determined by the location of the patient's pain. For pain of the extremities or trunk the coordinates were 9 mm posterior, 10 to 12 mm lateral, and 2 to 5 mm dorsal to the midpoint of the anterior commissure–posterior commissure (AC-PC) line. Coordinates for facial pain were 8 mm posterior, 8 mm lateral, and 3 to 5 mm dorsal to midpoint of the AC-PC line. These are the coordinates of Adams and Hosobuchi (*19*). Immediately after insertion of the first electrode (*20*), but before stimulation was begun, a second sample of CSF was obtained. Stimulation was then begun and adjusted to produce maximum relief of pain as reported by the patient. Stimulation parameters arrived at through this method were similar to those in previous studies (*4, 14*). This stimulation was continued for 15 minutes, and a third sample of CSF was obtained. The second electrode was then implanted. Immediately after implantation of the second electrode a fourth sample of CSF was obtained. Stimulation was then begun, adjusted as before, and continued for 15 minutes. A final sample of CSF was obtained after this stimulation. All samples were immediately frozen on dry ice and stored at −90°C until being assayed for β-ELI and β-lipotropin (*21*).

The results of the effects of PVG and VPT stimulation on CSF β-ELI are shown in Fig. 1. Two groups are represented: those patients who received PVG stimulation before VPT stimulation and those patients who received either VPT stimulation before PVG stimulation or who received bilateral VPT stimulation. The latter two (control) groups differ slightly, but both had VPT stimulation first (and thus the first four of five CSF samples were drawn under identical conditions); therefore, the data on them were combined for analysis. Two-way analysis of variance with repeated measures revealed that neither the difference between groups nor the interaction between groups and time was statistically significant. Changes in the level of β-ELI over time (collapsed across groups), however, were significant [$F(4,40) = 13.77$, $P < 0.01$]. Subsequent analysis of this result with a *t*-test with repeated measures demonstrated that β-ELI at each time point significantly exceeded control levels ($P < 0.01$ in each case) (*22*). Therefore, as in previous studies, these results demonstrate significant increases in CSF β-ELI in patients receiving electrical stimulation of the brain for chronic pain (*2–4*).

β-Lipotropin in CSF increased only 50 percent on average after contrast infusion and did not change further with

Table 1. Patient characteristics and electrode targets. Abbreviations: FLB, failed low back (includes patients with epidural fibrosis or arachnoiditis); LB, low back; LLE, left lower extremity; and LUE, left upper extremity.

Initials	Sex	Age	Etiology of pain	Location of pain	Electrode	
					1	2
P.V.	M	43	FLB; postcordotomy dysesthesia	LB and LLE	PVG	VPT
G.Q.	M	54	FLB; failed cervical laminectomy	Cervical; LB	PVG	VPT
C.F.	M	43	Causalgia	LUE	PVG	VPT
O.C.	F	55	Peripheral deafferentation	LLE	PVG	VPT
J.L.	F	50	Peripheral deafferentation	Chest	PVG	VPT
J.V.	F	56	Anesthesia dolorosa	Right face	PVG	VPT
P.E.	F	40	Atypical facial pain	Right face	PVG	VPT
G.B.	F	43	FLB	LB and LLE	PVG	VPT
J.W.	M	70	Thalamic pain	Hemicorporal below neck	VPT	PVG
B.P.	F	66	Peripheral deafferentation	Bilateral perianal	VPT	VPT
M.V.H.	F	63	Atypical facial pain	Left face	VPT	VPT
G.Q.	M	55	FLB; failed cervical laminectomy	Cervical; LB	VPT	PVG

PVG or VPT stimulation. None of the β-lipotropin alterations reached statistical significance. Furthermore, Pearson product-moment correlation analysis of the β-ELI and β-lipotropin results did not reveal a significant correlation between the effects of stimulation on these peptides ($r = 0.06$). Our control levels and percent increases of β-ELI are similar to those reported previously. That our baseline values are slightly lower than those in a previous study (*4*) is probably the result of increased specificity of the assay. However, the data gathered in this experiment, in which a CSF sample was collected after contrast infusion but before stimulation was begun, lead to a very different conclusion than that suggested previously. As shown by Fig. 1, the increase in β-ELI occurred before stimulation began. Moreover, β-ELI did not increase further after stimulation but returned toward normal. Although different contrast agents were used in previous studies (*23*), findings discussed below suggest that this difference has no effect on the β-ELI results.

Anatomic specificity of the effect of PVG stimulation on CSF β-ELI was previously suggested by the observation that patients receiving electrical stimulation of the posterior internal capsule did not have significant elevations of CSF β-ELI (*4*). However, in the present study patients who received stimulation of the VPT before PVG stimulation or stimulation only of the VPT had not only the same qualitative and quantitative elevation in β-ELI but also the same temporal pattern of elevation. These results strongly indicate that CSF β-ELI elevations occur prior to, and independent of, electrical stimulation of the PVG. We recently reported similar β-ELI elevations in lumbar CSF of patients receiving only routine metrizamide myelography by lumbar puncture (*24*).

Several explanations could account for these results. First, because stress has been reported to elevate CSF β-ELI (*25*), it is possible that the surgical procedure alone is sufficiently stressful to elevate β-ELI. Second, electrical stimulation of the brain might result in elevation of CSF β-lipotropin that, because of the assay cross-reactivity, would appear as β-ELI. Third, the contrast medium might induce the release of β-endorphin by direct irritation of periventricular tissues. Finally, the contrast medium might directly interfere with the assay, thereby falsely causing the appearance of elevated β-ELI. In vitro assays cause us to favor the final explanation. Using minute quantities of any of four commonly used contrast agents [Amipaque, Renograffin, Conray, or Pantopaque (*26*)], we can accurately reproduce the in vivo results reported above (*27*).

Several other observations support our suggestion that the contrast agent, not PVG stimulation or β-lipotropin, is responsible for the observed increases in CSF β-ELI. First, additional assays in one patient (J.V.) indicate that CSF β-ELI is elevated to levels similar to those reported above within 1 minute of contrast infusion. Such a rapid increase would be inconsistent with a physiological effect of stimulation (since no stimulation occurred), an irritative effect, and even a stress effect (since the control sample was taken just 1 minute before this sample and the surgical preparation had already been completed). Second, although one patient (J.W.) required general anesthesia, which should have reduced the conscious stress component, his CSF β-ELI followed the same pattern as all the others. Third, a localized cutaneous infection made it necessary to remove the electrodes from one patient (G.Q.) after approximately 4 months of successful pain control. When reimplanting his electrodes we reversed the sequence of implantation. His CSF β-ELI

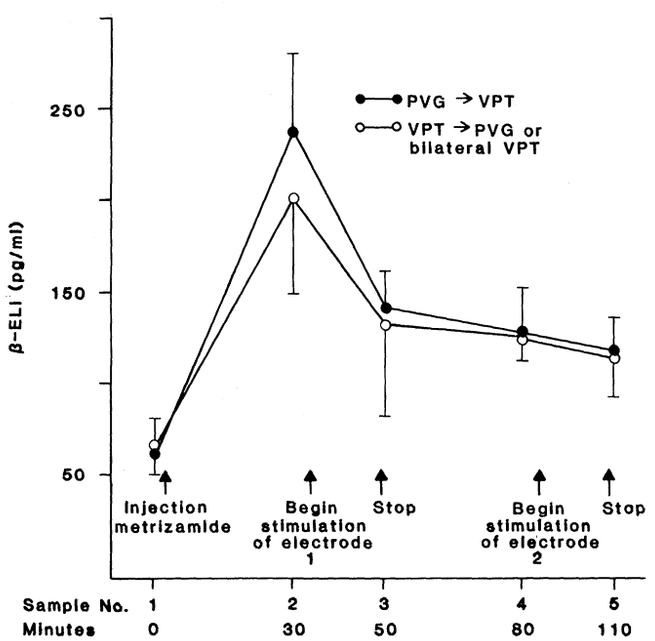

Fig. 1. Concentration of CSF β-ELI in patients receiving electrical stimulation of the PVG before stimulation of the VPT and in patients stimulated in the reverse order or given bilateral stimulation of the VPT. Collection of CSF samples and times of specific manipulations are indicated. Values are means ± standard errors.

levels were virtually identical in the two procedures. Fourth, β-lipotropin in the same CSF samples was elevated only insignificantly after contrast infusion, and was not correlated with β-ELI within or between samples. Finally, Martin-Rodriguez and Obrador (*5*) reported no increase—or, at best, inconsistent changes—in CSF "endorphin-activity" after PVG stimulation in samples drawn from three patients with Ommaya reservoirs (and therefore not subject to interference from contrast infusion) (*28*). Hosobuchi *et al.* (*29*) reported significant elevations of β-ELI in Ommaya CSF samples from three patients receiving PVG stimulation who had been treated with tryptophan and who were not tolerant of the analgesic effects of PVG stimulation. Patients who were tolerant of stimulation (and not tryptophan-treated) did not have stimulation-induced elevations. However, tryptophan-treated patients are not comparable to drug-free patients, and thus the relevance of this observation to our results and those of Martin-Rodriguez and Obrador is unclear.

In summary, we have demonstrated increases in CSF β-ELI after brain stimulation that are similar in magnitude to those reported previously. The number of patients, location and etiology of pain, surgical procedures, stereotaxic coordinates, and stimulation parameters in our study and the previous studies were all similar. Furthermore, all the patients were experiencing 50 to 100 percent relief of their pain and all received iodine-laden contrast medium for ventriculography. It appears that a direct effect of the contrast medium on the assay, not electrical stimulation, is responsible for the elevated β-ELI. It should be noted that these findings do not reflect potential changes in CSF methionine-enkephalin–like immunoreactivity after stimulation. Such changes have been reported in chronic pain patients receiving PVG stimulation. Since the radioimmunoassay data were replicated by bioassay in this study, the results presumably are reliable. Furthermore, our data do not enable us to state unequivocally that PVG stimulation does not increase CSF β-endorphin. It is possible that small increases did occur but were masked by the larger contrast-mediated interference with the β-ELI assay. What can be suggested, however, is that neither our results nor the previous observations support the hypothesis that increases in CSF β-endorphin occur after PVG stimulation alone.

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18. In this procedure metrizamide (2 to 6 ml), a radio-opaque dye (Amipaque, Winthrop Laboratories), is infused into the third ventricle through the catheter. Anterior-posterior and lateral x-rays are then taken to identify landmarks.
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21. To measure β-ELI we followed the procedure included with the kit (No. NEK-003, New England Nuclear). The assay has 50 percent cross-reactivity with β-lipotropin but less than 0.01 percent with α-endorphin, leucine-enkephalin, methionine-enkephalin, and α-melanocyte–stimulating hormone. β-Lipotropin was also assayed by using the procedure included with the kit (No. NEK-011, New England Nuclear). This assay has insignificant cross-reactivity with β-endorphin.
22. The rationale for this statistical approach was discussed by B. J. Winer [*Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), p. 89] and G. Keppel [*Design and Analysis: A Researcher's Handbook* (Prentice-Hall, Englewood Cliffs, N.J., 1973), p. 93].
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