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Appearance of β-endorphin-like immunoreactivity in human ventricular cerebrospinal fluid upon analgesic electrical stimulation

(β-endorphin/analgesia)

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ABSTRACT **β-Endorphin-like immunoreactivity in human ventricular cerebrospinal fluid was measured with a specific radioimmunoassay. The subjects were undergoing a surgical procedure for relief of chronic intractable pain. This procedure involved the focal stimulation of a medial thalamic site adjacent to the wall of the third ventricle. Samples were collected before and during the analgesic stimulation. No β-endorphin-like immunoreactivity could be detected prior to stimulation, suggesting that baseline levels are below 25 fmol/ml of cerebrospinal fluid. Electrical stimulation led to substantial increases (13- to 20-fold) in immunoreactive material in every subject. These results suggest that β-endorphin-like material can be released into the ventricular system and may contribute to the pain blockade that results from periventricular stimulation.**

The isolation and characterization of β-endorphin from camel (1), sheep (2), and human (2, 3) pituitaries rapidly led to its identification as an endogenous opioid (1–4). Administration of this 31-amino-acid peptide to laboratory animals results in phenomena like those associated with opiates, including naloxone-reversible analgesia (5–10), tolerance (8–11), and dependence (8, 11). A number of other phenomena have been described, including dramatic motor effects (12, 13) and the production of limbic seizures (14). β-Endorphin has also been administered to several groups of human subjects. Some of its effects include pain blockade upon intravenous (15) and intraventricular (16) administration and suggestion of alterations in affect and mood in manic, depressed (17), and schizophrenic (17) patients. Most of these reported effects are long-lasting, with durations ranging up to several days.

While it was readily apparent that the pharmacological effects of β-endorphin were at least partially mediated by the central nervous system, it was not clear whether the brain was primarily a target organ for pituitary release of the opioid, or whether it produced its own β-endorphin. During the past year a nonpituitary, brain β-endorphin system has been described (18–20). This system also exhibits β-lipotropin immunoreactivity (21, 22) and corticotropin (ACTH)-like immunoreactivity (20, 23), suggesting the existence in brain of the common 31,000 molecular weight precursor of β-lipotropin/corticotropin that has been described in pituitary (24, 25). The brain β-endorphin/corticotropin system is easily distinguished from enkephalin systems. It arises from a single cell group in the hypothalamic periarcuate region and projects long pathways into various brainstem structures, including the nucleus accumbens, basal septum, amygdala, periventricular thalamus, central grey, and locus coereleus. The functions of this system remain unknown and the potential role of brain β-endorphin as a neurotransmitter remains unexplored. We now report that electrical stimulation of periventricular medial thalamus in human patients leads to the release of β-endorphin-like immunoreactivity into the third ventricle.

Electrical stimulation of the brain has been shown to elicit analgesia in rat (26) and cat (27). This analgesia bears many of the characteristics of opiate action, including partial reversal with the opiate antagonist naloxone (28–30) and the development of tolerance to and cross-tolerance with morphine (31). However, these effects are only partial, suggesting a role of nonopiate as well as opiate mechanisms in this method of pain control. This work has been extended to the human clinical situation. Patients suffering from chronic intractable pain derive long-term relief from the stimulation of medial thalamic and periaqueductal sites (32, 33). Stimulation of a site in the vicinity of the posterior commissure, immediately adjacent to the ventricular wall at the level of the nucleus parafascicularis, has been found to combine excellent analgetic effectiveness with minimal side effects. The characteristics of the pain relief obtained from such stimulation have been described (32, 33). This stimulation-induced analgesia in man also appears to have an opioid component, because it has been shown to be naloxone reversible (34, 35) and to be accompanied by a rise in enkephalin-like opioid material in the cerebrospinal fluid (CSF) (36). Because the stimulation site in humans is homologous to a region in rodents particularly rich in β-endorphin activity, we have examined the effect of its stimulation on β-endorphin immunoreactivity in CSF.

METHODS

Surgery and Collection of Samples. The subjects (5 males) were part of a series of 30 patients suffering from severe, intractable pain who underwent stereotaxic surgery for electrode implant in the periventricular gray region. The surgical procedure and its overall clinical success for the first 8 patients have been described (33). The first stage of the surgery consisted of stereotaxically implanting a multipolar electrode at a site near the posterior aspect of the third ventricle medial to the nucleus parafascicularis and in close proximity to the posterior commissure (Schatelbrand and Bailey coordinates: Fp = 10, Ht = 0, Lat = 2–5). During this stage, the patient was under local anesthesia and was able to report on the intensity of pain before, during, and after stimulation. The site was chosen empirically, such that its stimulation produced the most potent relief of pain with the least side effects. The electrode was then secured to the skull but remained externalized for several days to permit further testing. The second stage was then carried out with the patient under general anesthesia. At that point, the electrode was internalized and connected to a Medtronics receiver im-

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Abbreviation: CSF, cerebrospinal fluid.

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Table 1. Electrical stimulation increases β-endorphin-like immunoreactivity in ventricular CSF (results given in fmol of β-endorphin equivalents per ml)

Patient	Baseline before stimulation	Time after onset of stimulation 0–5 min	5–10 min	10–15 min	15–20 min
Nm	<25	250	450	500	
B	<25	750	300	250	325
S	<25	Not collected	250	400	
Ns	<25	575	270	270	
LB	<25	350	325	350	
Mean ± SEM	<25	481.25 ± 112.44	319.0 ± 35.16	354.0 ± 45.45	

planted in the chest pectoralis region. A Medtronics stimulator was then given to each patient to allow him to self-administer the current at the desired parameters. Several days of work with the patient were used to select the specific current parameters (amplitude, frequency, etc.) for each subject that produced good analgesia with little or no discomfort.

All patients studied here reported significant or complete pain relief with periventricular stimulation. In these and other patients, the procedure has proven useful for several months or years.

In the present study, the CSF samples were collected in stage 1 via an intraventricular catheter introduced into the third ventricle so that we could inject a Conray dye and visualize the commissures for the purposes of the stereotaxic implant. Four to five samples of ventricular fluid (approximately 3 ml each) were withdrawn. The first sample was obtained prior to any electrical stimulation and constituted the baseline control. All the other samples were collected at intervals of approximately 5 min during the course of electrical stimulation. Some variability in the procedure was dictated by the patient's response and the necessity to adjust the stimulation parameters. Still, all patients exhibited significant analgesia to the stimulation during the procedure as determined from testing their responsiveness to acute pain and their reports of chronic pain.

The CSF samples were frozen immediately after being collected. Prior to assay they were immersed in a boiling water bath to halt the possible action of degradative enzymes; the samples were kept frozen at −20°C until the time of assay.

Radioimmunoassay of β-Endorphin. Antiserum to β-endorphin was obtained by injecting rabbits with a carbodiimide-coupled IgG-conjugated β-endorphin antigen. The radioimmunoassay was carried out in a sodium phosphate buffer (0.01 M, pH 7.5/0.1 g of bovine serum albumin per 100 ml/0.9% NaCl), in a total volume of 0.25 ml. The incubations were at 4°C for 48 hr. Bound and unbound ligand were separated by addition of 0.5 ml of charcoal slurry. β-Endorphin was radiolabeled with ^{125}I by the chloramine-T method, yielding a specific activity of over 100 Ci/mmol. The assay can detect around 2.5 fmol. β-Lipotropin does crossreact with this antiserum, but is 1/6th–1/10th as effective on a molar basis. The antibody does not crossreact with β-melanotropin, corticotropin, or methionine-enkephalin at concentrations of 100 nM. The effect of artificial CSF on the binding and displacement of labeled β-endorphin was examined. It was determined that 100 μl of artificial CSF per tube did not cause any significant changes in the control value or in the sensitivity and slope of the β-endorphin dilution curve. Therefore 100 μl of sample was assayed in triplicate and compared to control samples containing the same volume of artificial CSF.

RESULTS

Table 1 summarizes the effects of electrical stimulation of β-endorphin-like immunoreactivity in each of the samples. As can be readily seen, baseline samples did not exhibit any detectable immunoreactivity. Because we assayed 0.1 ml, and the smallest detectable amount is 2.5 fmol, this means that baseline levels in these subjects were below 25 fmol of β-endorphin (or its equivalent immunoreactivity) per ml of CSF. However, analgetic electrical stimulation led to a substantial rise in the levels of immunoreactive material at each time point and in each subject. The amounts measured ranged between 319 ± 35 and 481 ± 45 fmol of β-endorphin equivalents per ml, suggesting 13- to 20-fold increase above the maximum possible baseline levels (25 fmol/ml). Because the antibody used in this study exhibits partial crossreactivity with β-lipotropin, it is possible that the rise seen could be due to β-lipotropin release. However, this would require an even more dramatic rise in β-lipotropin levels in the CSF, because this molecule is 1/6th–1/10th as active in binding the antibody.

DISCUSSION

The increase in β-endorphin-like immunoreactivity observed in this study suggests that brain β-endorphin is releasable with electrical stimulation—an important criterion towards identifying it as a putative neurotransmitter. Furthermore, these results point to a potential role of brain β-endorphin in the production of stimulation analgesia in human subjects. While it is probable that other opiate-like factors would be released upon periventricular stimulation, the changes seen in enkephalin-like material under the same conditions are of much smaller magnitude (less than 2-fold, cf. ref. 36).

Because the intraventricular infusion of β-endorphin in humans leads to analgesia, it is conceivable that the liberation of endogenous β-endorphin-like material in the ventricular system by our procedure could be partially responsible for the long-lasting analgesia observed in our subjects. Finally, because β-endorphin-like material has been detected under the stimulated conditions, it is conceivable that more sensitive methods, possibly using extraction and concentration steps, may lead to identification and quantification of β-endorphin in normal states.

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