Elevated Immunoreactive $\beta$-endorphin Level in Ventricular Fluid after Analgesic Electrical Stimulation of Posteromedial Hypothalamus

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Abstract

Immunoreactive $\beta$-endorphin ($\beta$-EP) in the ventricular fluid of six carcinomatous patients was measured using a specific radioimmunoassay. The subjects were undergoing a surgical procedure for relief of chronic intractable pain. This procedure involved the focal stimulation and coagulation of the posteromedial hypothalamus. Samples of ventricular fluid were collected before and after the stimulation and serially after the coagulation. Prior to stimulation, $\beta$-EP-like immunoreactivity ($\beta$-EP-LI) was below 200 pg/ml. In all of the six patients with pain relief, electrical stimulation led to a marked increase in immunoreactive $\beta$-EP. In three patients $\beta$-EP levels remained high after electrical coagulation for 6-24 hrs. These results suggest that $\beta$-EP-like material, released into the ventricular fluid, may contribute to the initial pain blockade that results from stimulation and coagulation of the posteromedial hypothalamus.

Electrical stimulation of the brain has been shown to elicit analgesia in rat (Raynolds 1969, Mayer et al., 1971), cat (Liebeskind et al., 1973), and monkey (Goodman 1975). This analgesia bears many of the characteristics of opiate action, including partial reversal with the opiate antagonist naloxone (Akil et al., 1976, Oliveras 1977) and the development of tolerance to and cross-tolerance with morphine. This work has been extended to the human clinical situation. Patients suffering from chronic intractable pain derive long-term relief from the electrical stimulation of medial thalamic and periaqueductal sites (Hosobuchi et al., 1977, Richardson et al., 1977). Intraventricular administration of human $\beta$-endorphin ($\beta$-EP) in humans produce a prolonged state of analgesia (Oyama et al., 1982). The current research was undertaken to study the relationship between the analgesia produced by stimulation of the posteromedial hypothalamus and the $\beta$-EP concentrations in ventricular fluid (Mayanagi et al., 1978).

Materials and Methods

1. Radioimmunoassay (RIA) for $\beta$-EP
   Antiserum preparation
   Immunization against synthetic $\beta$-EP was carried out in the rabbit. Two rabbits were injected in the

Received July 12, 1983
dermis of the back at multiple sites with the $\beta$-EP (100 $\mu$g) emulsified with complete Freund's adjuvant and were boostered with $\beta$-EP at monthly intervals. Sera containing antibodies were vialled in 0.2 ml aliquots and kept frozen at $-60^\circ$C.

Iodination of $\beta$-EP

$\beta$-EP was iodinated with Na$^{125}$I by the lactoperoxidase method (Miyachi et al., 1972), and purified by chromatography on Biogel P-10, yielding a specific activity of approximately 100 $\mu$Ci/$\mu$g.

RIA procedure

Standard solutions, samples for testing and RIA-tubes were kept on ice during assay pipetting. The RIA of $\beta$-EP was carried out in phosphate buffer saline (1/15 M, pH 7.4) containing 0.1% bovine serum albumin and 1,000 KIE Trasylol. Antiserum (finally diluted to 1 : 10,000), iodinated $\beta$-EP and 1-10,000 pg of standard $\beta$-EP or samples were added to tubes in a total volume of 0.5 ml and mixed vigorously.

After the incubation at 4°C for 24 hrs., 0.5 ml of 0.5% dextran-coated charcoal solution was added to the tubes. The contents of each tube were mixed, incubated at 4°C for 15 minutes and centrifuged at 2,500 rpm for 15 minutes. The radioactivity of the supernatant was counted with a well type gammaspectrometer.

When the cerebrospinal fluid (CSF) levels of $\beta$-EP were measured by RIA, $\beta$-EP free CSF which was obtained by incubation at 37°C for 24 hrs. was added to the tubes containing standard $\beta$-EP. Our data showed that there was no immunoreactive $\beta$-EP left when CSF with a high titer of $\beta$-EP-like immunoreactivity ($\beta$-EP-LI) after stimulation and coagulation was incubated at 37°C for 24 hrs.

2. Samples

CSF samples were collected by lumbar tap from ten control subjects who had no endocrinological or psychological abnormalities and were stored at $-20^\circ$C until the assay of $\beta$-EP. Ventricular fluid was collected from six patients with carcinoma undergoing stereotactic surgery to the posteromedial hypothalamus to relieve intractable pain (Table 1). The outline of this surgery is given below.

After demonstration of the three ventricles, a fine concentric bipolar needle electrode, 0.8 mm in outer diameter with an interpolar distance of 0.5 mm, was stereotactically inserted under local anesthesia through a frontal burr hole into the target point. The coordinate of the target point was 2 mm below the midcommissural point and 2 mm lateral from the wall of the third ventricle. Manifestations of good sympathetic response, which are elevated blood pressure, increased pulse rate, respiratory arrest followed by hyperpnea or tachypnea, mydriasis, and flushing of the face, could be obtained by high frequency stimulation (50 Hz, 10 V for 10 seconds) at this point, and electrocoagulation with high frequency current (1 megaHz, 2-3 Watt for 1 minute) was performed in the area where the symptoms mentioned above were most prominent.

Samples of ventricular fluid were collected from the ventricular catheter inserted to perform intraoperative ventriculography, at the onset of the surgery prior to ventriculography, at the insertion of the electrode, at stimulation, at the end of the 10 second stimulation period. In three patients, ventricular fluid was collected at the end of the one minute coagulation procedure and serially after the coagulation until 6-96 hrs. The samples were frozen and stored at $-20^\circ$C.

3. Gel chromatography

Chromatographic separation of human $\beta$-EP and human $\beta$-lipotropin ($\beta$-LPH) was conducted on a 0.9 x 90 cm column packed with Sephadex G-50 superfine, equilibrated with phosphate buffer saline (1/15 M, pH 7.4) at 4°C. In an attempt to determine the excluded position of $\beta$-EP and $\beta$-LPH in this column, about 100,000 cpm of iodinated $\beta$-EP and $\beta$-LPH were applied to the column and eluted with phosphate buffer saline. Two ml of eluate was collected and the radioactivity in each fraction was measured with a gammaspectrometer. 10 ml of lyophilized ventricular fluid, a mixture of samples obtained after stimulation and coagulation, which was dissolved with 250 $\mu$l of distilled water was applied to the column and eluted with phosphate buffer saline. Two ml of eluate was collected and the radioactivity in each fraction was measured with a gammaspectrometer. 10 ml of lyophilized ventricular fluid, a mixture of samples obtained after stimulation and coagulation, which was dissolved with 250 $\mu$l of distilled water was applied to the column and eluted with phosphate buffer saline. Two ml of eluate was collected and the radioactivity in each fraction was measured with a gammaspectrometer. 10 ml of lyophilized ventricular fluid, a mixture of samples obtained after stimulation and coagulation, which was dissolved with 250 $\mu$l of distilled water was applied to the column and eluted with phosphate buffer saline. Two ml of eluate was collected and the radioactivity in each fraction was measured with a gammaspectrometer.

Table 1. Clinical Summary of the Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Etiology of pain</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>F</td>
<td>Lymphoepithelioma</td>
<td>Rt. posteromedial hypothalamotomy</td>
</tr>
<tr>
<td>B</td>
<td>54</td>
<td>F</td>
<td>Renal cancer</td>
<td>Rt. posteromedial hypothalamotomy</td>
</tr>
<tr>
<td>C</td>
<td>26</td>
<td>F</td>
<td>Rectal cancer</td>
<td>Lt. posteromedial hypothalamotomy</td>
</tr>
<tr>
<td>D</td>
<td>43</td>
<td>M</td>
<td>Malignant melanoma</td>
<td>Rt. posteromedial hypothalamotomy</td>
</tr>
<tr>
<td>E</td>
<td>57</td>
<td>M</td>
<td>Seminoma</td>
<td>Rt. posteromedial hypothalamotomy</td>
</tr>
<tr>
<td>F</td>
<td>60</td>
<td>F</td>
<td>Pancreas cancer</td>
<td>Rt. posteromedial hypothalamotomy</td>
</tr>
</tbody>
</table>
4. Chemicals

Human β-LPH was kindly provided by C. H. Li (San Francisco, USA). Human corticotropin (ACTH), β-melanocyte stimulating hormone (β-MSH), human luteinizing hormone (LH) and human follicle stimulating hormone (FSH) were provided by NIAMDD (by courtesy of NIH). Sheep β-EP was purchased from the Peninsula Lab. (Montreal, Canada), β-EP, α-EP, substance P and neurotensin from Protein Research Foundation (Osaka, Japan), Trasylol from Bayer (Leverkusen, Germany) and ethylenediamine tetraacetic acid (EDTA) from Katayama Chemicals (Osaka, Japan).

Results

Specificity of the human β-EP antiserum

The binding of 125I-β-EP to antibody exhibited a dose-related inhibition with added unlabeled human β-EP in the range of 10–10,000 pg (Fig. 1). The assay sensitivity, defined as the minimal detectable amount, was 20 pg/tube.

Fig. 1. Specificity and sensitivity of β-endorphin radioimmunoassay.

Fig. 1 shows that human β-LPH and sheep β-LPH could produce a parallel dose-related inhibition of binding in the assay. The inhibition curves of human β-LPH and sheep β-LPH were shifted to the right of the human β-EP curve so that the cross-reactivities were 48.9% and 19.9% on a molar basis, respectively. α-EP, i.e. β-EP-(1–16), also inhibited the binding and the cross-reactivity to inhibit 50% of the antibody binding of labelled peptide was less than 0.4%.

The antiserum showed no cross-reaction with methionine-enkephalin, β-MSH, substance P, somatostatin, neurotensin, prolactin, TSH, GH, LH or FSH measured in the assay in the amounts of 0.1–1,000 ng/tube.

Gel chromatography

To show that β-EP really exists in human CSF and to see whether it corresponds to β-EP-LI, we performed chromatography of human β-EP and human β-LPH using Sephadex G-50 superfine. As shown in Fig. 2, chromatography of purified 125I human β-EP and 125I human β-LPH showed that their peak radioactivities were observed separately at the 34th and 23rd fraction, respectively. When the CSF extract was also subjected to gel chromatography on the same column and the β-EP content of each fraction measured by radioimmunoassay, one broad immunoreactive peak was found at the elution position corresponding to the radioactive peak of 125I-β-EP, suggesting the heterogeneity of this peak. Little immunoreactivity was found at the position corresponding to the human β-LPH peak (Fig. 2).

RIA

It was observed that the addition of β-EP free CSF to the tubes caused no significant changes in the sensitivity or the slope of β-EP standard curve. When aliquots of 50, 100 and 200 μl of human third ventricular fluid, containing a high titer of β-EP-LI, were measured in the β-EP RIA system, the inhibition of binding profile was parallel to the standard curve.

Values of β-EP-LI in human ventricular fluid upon analgesic electrical stimulation

When 100 μl CSF samples, collected by lumbar tap from ten control subjects with no endocrinological abnormalities, was measured directly, immunoreactive β-EP was not detected, suggesting that the normal
CSF levels of β-EP are lower than 200 pg/ml.

All of the six patients reported here (Table 1) showed complete or significant relief of pain following posteromedial hypothalamic stimulation. Fig. 3 summarizes the effect of electrical stimulation on the levels of β-EP-LI in the third ventricular fluid of six carcinomatous patients. Samples, obtained prior to any electrical stimulation, constituting the baseline control, were less than 200 pg/ml. β-EP-LI levels in the six patients increased significantly to 628.6 ± 169.0 pg/ml (mean ± SEM) at the end of the stimulation period and became as high as 463.0 ± 197.4 pg/ml at the end of the coagulation period.

In one patient (D) β-EP-LI levels in the third ventricular fluid were measured till 48 hrs. after the cessation of coagulation, and they were not detected at 12 hrs., 24 hrs. or 48 hrs. after the cessation. In another patient (E) measured till 96 hrs. after the cessation of coagulation, β-EP-LI levels were not detectable at 72 hrs. or 96 hrs. after the cessation, although in both patients relief of pain continued (Fig. 3).
Discussion

When immunizing against synthetic human \( \beta \)-EP, the immunogen used was prepared by coupling human \( \beta \)-EP to large molecules. In our study, synthetic human \( \beta \)-EP was not conjugated with any carrier agent in order to elicit specific antibody production. The peptide was injected into the rabbits, mixed with only complete Freund’s adjuvant. The cross-reaction of this antiserum with human \( \beta \)-LPH was 48.9\% on a molar basis, which is less than that of antisera to \( \beta \)-EP-albumin previously reported by several groups (Nakai et al., 1978, Hollt et al., 1979, Malizia et al., 1979, Jeffcoate et al., 1978, Guillemin et al., 1977), while Akil et al., Wardlaw and Franz, Ross et al., Li et al., and Matsumura et al., showed that their antisera produced by immunizing \( \beta \)-EP-albumin conjugate had the ability to discriminate \( \beta \)-EP partially from \( \beta \)-LPH (4.5-30\% molar cross-reactivity).

The minimal detectable quantity of human \( \beta \)-EP in our study was 20 pg/tube, which implies that the assay sensitivity was not as good as that reported by others. The minimal detectable quantities in RIA by Guillemin et al., and others (Akil et al., 1978, Li et al., 1977, Liotta et al., 1979, Nakai et al., 1978, Jeffcoate et al., 1978, Hollt et al., 1979, Domschke et al., 1979 and Malizia et al., 1979) are reported 1-100 pg/tube.

Human \( \beta \)-EP-LI could not be detected in CSF obtained by lumbar puncture from ten control subjects with no endocrinological or psychological abnormalities, or in the third ventricular fluid from six patients with intractable pain (Fig. 3). Human \( \beta \)-EP-LI levels in CSF have been reported as follows. Akil et al., detected no human \( \beta \)-EP-LI in the ventricular fluid of patients with intractable pain or in the CSF of normal men obtained by lumbar puncture. Jeffcoate et al., selected ten patients (mean age 50, range 21-71) with neurological symptoms mainly due to discal hernia and measured the immunoreactive \( \beta \)-EP levels in their CSF samples and found them to be 72 ± 7 pmol/l (252 ± 24.5 pg/ml).

In the gel filtration pattern of CSF with high titer \( \beta \)-EP-LI after stimulation and coagulation, the major peak was eluted at the position of authentic \( \beta \)-EP and a small peak was eluted at the position of authentic \( \beta \)-LPH. Similar peaks have been reported in the CSF of a patient with hyperprolactinemia (Jeffcoate et al., 1978). In another report of elution profiles obtained by gel filtration of CSF extracts of four patients without endocrinological abnormalities, one patient with Nelson’s syndrome, and one patient under glucocorticoid therapy, two major peaks corresponding to the positions of void volume and authentic \( \beta \)-LPH and one minor peak corresponding to the position of authentic \( \beta \)-EP were reported (Nakao et al., 1980). The difference in the elution pattern of CSF \( \beta \)-EP-LI may be considered to be due to the differences in the disease and therapy of each patients.

\( \beta \)-EP-LI in human ventricular fluid was not detectables before electrical stimulation in six patients suffering from intractable pain of malignancy, but it was elevated to 300-1,300 pg/ml after the electrical stimulation. In three patients whose ventricular fluid was obtained after electrical coagulation, \( \beta \)-EP-LI maintained high levels for 6-24 hrs. after coagulation, and these there-after returned to undetectable levels. By posteromedial hypothalamus coagulation, all of the six patients showed significant relief from pain for 6 to 12 months. The data reported here suggest a relationship between \( \beta \)-EP levels in ventricular fluid and relief of pain immediately after posteromedial hypothalamus coagulation. As for the longer duration of the analgesic effect obtained by electrical coagulation, however, there seems to be little relation, and further studies regarding this point must be
undertaken.

Acknowledgements

We are grateful to Prof. Dr. M. B. Lipsett for his review of the manuscript. The gifts of human LH and FSH standards from the NIAID, Bethesda, Maryland, USA, and of β-LPH from Prof. C. H Li, University of California, San Francisco, USA are also gratefully acknowledged.

References


