Volume 57, No. 1
July 1982

THE JOURNAL OF Anesthesiology
THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

Anesthesiology
57:1–4, 1982

Endorphin Levels in Cerebrospinal Fluid of Patients with Postoperative and Chronic Pain

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The authors measured endorphin levels in the cerebrospinal fluid (CSF) of 12 patients with chronic pain due to lumbar disc syndrome and eight patients with acute postoperative pain. These were compared with CSF endorphin levels in 20 control patients with no history of pain. Endorphins were extracted by adsorption to a synthetic resin (Amberlite XAD-2®), eluted with methanol, and assayed using the electrically stimulated mouse vas deferens. Results were expressed as methionine-enkephalin (Met-E) equivalents, which was the standard in the bioassay. The CSF endorphin level was 0.42 ± 0.07 pmol/ml (mean ± SE) in the postoperative group, 1.44 ± 0.2 pmol/ml in the chronic pain group, and 4.36 ± 0.89 pmol/ml in the control group. CSF endorphin levels in the two pain groups differed significantly from both the control group and each other. These results suggest a correlation between pain levels and endorphin concentration in the CSF; however, in the acute postoperative pain group the influence of other factors such as anesthesia or surgical stress cannot be evaluated. (Key words: Cerebrospinal fluid: endorphins. Pain: postoperative. Polypeptides: endorphins.)

THE ANALGESIC EFFECTS of endorphins when administered to both humans and other mammals are well-documented.[1–4] Thus, a potential role of these endogenous opiates in pain regulation has been suggested.[5] However, the role of endorphins in different pain states has not yet been elucidated.[6,7] Two methods have been used to determine endorphin activity in humans: the response to the administration of naloxone, and the quantitative determination of endorphins in cerebrospinal fluid (CSF). The latter method is based upon the assumption that CSF endorphin levels will reflect endorphin activity in the central nervous system (CNS). Altered levels of endorphins in the CSF of patients with different pain syndromes have been reported recently,[7,8] and variable levels of endorphins have been related to states of hyper- and hypoalgesia in humans.[5] In chronic pain, a decrease in CSF endorphin levels has been reported,[7] while in acute postoperative pain, no measurement of CSF endorphin levels have been described.

The authors' aim in the present study was to determine CSF endorphin levels in two groups of patients: one group with acute postoperative pain after high abdominal or thoracic surgery, and another group with chronic pain due to lumbar disc syndrome. These were compared to a control group of patients with no previous history of pain.

Materials and Methods

PATIENT SERIES

Samples of CSF were obtained from patients hospitalized in the C.S.V. Arrixaca Hospital, Murcia, Spain. Informed consent for the procedure was obtained from each patient. All lumbar punctures were done with the patient in a lateral decubitus, between 1300 and 1500 h. None of the patients was taking narcotic analgesics, non-narcotic analgesics, or tranquilizers at the time of CSF sampling. The subjects were grouped as follows, the main selecting criteria being their pathology as indicated below and the presence or absence of pain: 1)

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Received from the Department of Pharmacology, Murcia University School of Medicine, and the Department of Anesthesiology, C.S.V. Arrixaca Hospital, Murcia, Spain. Accepted for publication November 30, 1981. Presented in part at the 16th International Congress of Therapeutics, Porto, Portugal, October 4–8, 1981.

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0003-3022/82/0700/0001 $00.70

TABLE 1. CSF Endorphin Levels in the Control Group of Patients Without Pain

Patient Number	Age	Sex	CSF Endorphin Levels (pmol/ml)*
1	22	F	5.60
2	36	F	1.10
3	39	F	16.00
4	39	F	3.30
5	42	F	3.70
6	45	F	0.90
7	49	F	5.10
8	50	F	0.95
9	50	F	0.30
10	55	F	4.60
11	33	M	2.30
12	35	M	3.50
13	49	M	5.00
14	52	M	6.60
15	53	M	13.00
16	59	M	3.40
17	60	M	0.50
18	60	M	1.02
19	60	M	4.60
20	64	M	5.70
MEAN	47		4.36
SE	2		0.89

* Expressed as equivalents of methionine-enkephalin.

The control group was composed of 20 patients without a history of pain. CSF was obtained in the process of administering subarachnoid anesthesia in order to perform transurethral prostatic resections (n = 10) or uterine curettage (n = 10). 2) The postoperative group with acute pain was composed of eight patients following high abdominal or thoracic surgery. CSF was obtained two hours after recovery from anesthesia when a lumbar puncture was performed in order to administer subarachnoid morphine. Preoperative and intraoperative anesthetic drugs administered to these patients were as follows: 10 mg diazepam, im, the night before; 2.5 mg droperidol + 0.05 mg fentanyl (Innovar) + 0.75 mg atropine, iv, just before surgery; induction with 250 mg thiopental, iv; intubation after 1 mg/kg succinylcholine, iv; anesthesia maintained with N_2O/O_2 70 per cent and 0.05 fentanyl mg, iv in repeated doses; and pancuronium (initial doses of 0.1 mg/kg followed by successive doses of 2 mg, iv). 3) The chronic pain group was composed of 12 patients with lumbar disc syndrome of at least six-months duration. CSF was obtained before performing a diagnostic myelography.

PAIN MEASUREMENT

Pain was evaluated by means of a simple visual descriptive scale,[9] divided into five subjective levels of pain: none (grade 0); slight (grade 1); medium (grade 2); intolerable (grade 3); and excruciating (grade 4). All patients were evaluated for their pain level prior to lumbar puncture.

ENDORPHIN DETERMINATION IN CSF

A 5-ml CSF sample was frozen at −20° C immediately after collection and coded with a number which did not define the group to which the donor belonged. The following protocol was used for endorphin extraction from the coded samples. 1) Frozen samples were thawed and subsequently boiled for 5 min. 2) Samples were acidified by addition of 15 ml 0.1 N HCl. 3) 10 ml of Amberlite XAD-2® were added, agitated for 30 min, and the supernatant discarded. 4) The resin was washed in 35 ml distilled water × 3, and the supernatant discarded. 5) Endorphins were eluted from the resin by addition of a total of 20 ml methanol added in three steps (10, 5, and 5 ml). 6) Methanol was evaporated in a water bath kept at 40° C under a nitrogen atmosphere. 7) Dried samples were kept at 4° C until assay on the mouse vas deferens.

Samples were reconstituted with distilled water, and their opiate-like activity tested using the mouse vas deferens bioassay.[10] Male albino mice (Swiss Webster) 30–40 g were used; their vasa deferentia were removed and mounted for field stimulation (0.2 Hz, 0.5 ms, maximal voltage) in Krebs solution at 37° C. A 200-mg tension was applied initially and adjusted during a 30-min equilibration period. Contractions were recorded isometrically with a Grass® force displacement transducer (FT 03) on a Beckman® polygraph. For each assay the inhibitory effects of the CSF sample, as well as of the Met-E standard, were reversed by the addition of high concentrations of naloxone (1×10^{-7} to 1×10^{-6} M) to the bath.

All samples were reconstituted initially with 50 μl water, and 10 μl were tested for their inhibitory effect on the electrically induced contractions of the vas deferens. Based on the magnitude of the inhibitory response, the samples were diluted further or a larger volume of the undiluted sample (35 μl) tested; by this procedure we were able to determine the lower values of Met-E equivalents reported here. The results were interpolated onto a dose-response curve for Met-E (Serva) in the same preparation. Thus, the opiate-like activity present in our samples was expressed as picomole equivalents of Met-E per ml of CSF. This method allows recovery of 80–85 per cent of the enkephalin standard and has a range of sensitivity from 5.0×10^{-10} to 5.0×10^{-8} M.

The effects of droperidol and fentanyl on the endorphin assay were also studied. Each drug was added to 5 ml of saline at concentrations ranging from 2.0–200 ng/ml ($5.0–500 \times 10^{-9}$ M) for droperidol, and 0.1–10 ng/ml ($1.0–20 \times 10^{-9}$ M) for fentanyl, and processed following the procedure used for the CSF samples. Concentrations tested cover a wide range of plasma levels found at the time of CSF collection ($C_{t=2h}$), when up to 5 mg droperidol and 0.35 mg fentanyl citrate (0.15–0.35

TABLE 2. CSF Endorphin Levels in Postoperative Patients

Patient Number	Age	Sex	Type of Surgery	Degree of Pain	CSF Endorphin Levels (pmol/ml)*
1	44	F	Pleural decortication	2	0.30
2	68	F	Cholecystectomy	2	0.30
3	39	M	Porto-caval shunt	2	0.30
4	54	M	Cholecystectomy	2	0.60
5	55	M	Bilateral vagotomy	2	0.16
6	55	M	Thoracoplasty	2	0.50
7	59	M	Gastrectomy	1	0.80
8	63	M	Pyloroplasty and vagotomy	2	0.40
MEAN	54				0.42
SE	3				0.07

* Expressed as equivalents of methionine-enkephalin; the value for the patient number 5 is in the lower limit of detectability by our assay.

mg) were administered to the different patients during anesthesia (approximate plasma levels being 2–20 ng/ml for droperidol[11] and 0.5–1.0 ng/ml[12,13] for fentanyl two hours after administration of the drugs). Each drug concentration was tested at least twice.

STATISTICS

In analyzing differences between groups of patients, the Student's *t* test and analysis of variance were used.[14]

Results

Mean age of the control group was 47 ± 2 years and the mean endorphin level was 4.36 ± 0.89 pmol/ml of Met-E equivalents (table 1). When the female and male subgroups were compared, no significant differences were observed for either mean age or CSF endorphin levels. Thus, the control group was not divided according to sex or age.

In the acute pain group, the mean age was 54 ± 3 years (table 2) and all except one patient (number 7) had a high level of pain at the time of obtaining the sample. All patients had low levels of CSF endorphins, with a mean value of 0.42 ± 0.07 pmol/ml. This was significantly different from the control endorphin levels ($P < 0.01$). Since these patients had received a variety of drugs for anesthesia prior to sample collection (as described in Materials and Methods), we were concerned with the possible interference of some of these, such as fentanyl and droperidol, with the endorphin extraction and bioassay. Different concentrations of both drugs processed and assayed in identical conditions as the CSF samples showed no inhibitory effects on the assay.

In the chronic pain group, the mean age was 42 ± 3 years (table 3). This group also showed a significant decrease in endorphin levels ($P < 0.01$) as compared to controls with a mean of 1.44 ± 0.2 pmol/ml.

Discussion

The main finding of the study was a marked decrease in endorphin levels in the CSF of patients with acute and chronic pain. The CSF endorphin level in our control patients (4.36 ± 0.89 pmol/ml) was quite comparable to that reported by other investigators using both bioassay or radioreceptor (RRA) and immunoassays. For example, the value obtained by Akil *et al.*[15] in normal subjects was 3.42 ± 1.4 pmol/ml measured by RRA and mouse vas deferens bioassay. Similarly, Furui *et al.*[16] found a value of 2.6 ± 1 pmol/ml measured by RRA in 4 normal subjects using a different extraction procedure. Using RRA, Linstrom *et al.*[17] found a level of fraction II (probably equivalent to Met-E) of 2.5 ± 0.3 pmol/ml in normal subjects. Endorphin levels in CSF have been reported previously to be lower in patients with chronic pain syndromes.[7,8] In our study the group with chronic pain also showed low levels of CSF endorphins. However, the lowest levels of CSF endorphins were found in patients with acute postoperative pain, with a mean

TABLE 3. CSF Endorphin Levels in Chronic Pain Patients

Patient Number	Age	Sex	Degree of Pain	CSF Endorphin Levels (pmol/ml)*
1	33	F	1	2.60
2	34	F	2	0.40
3	48	F	2	1.00
4	56	F	3	0.80
5	61	F	2	2.80
6	32	M	2	2.00
7	34	M	2	0.90
8	35	M	2	0.50
9	36	M	2	0.83
10	37	M	1	2.22
11	50	M	2	1.83
12	55	M	1	1.40
MEAN	42			1.44
SE	3			0.2

* Expressed as equivalents of methionine-enkephalin.

value of 0.42 ± 0.07 pmol/ml. These low values were not due to interference of droperidol or fentanyl on the assay. Furthermore, the presence of these drugs in the CSF extracts could only potentiate the effects of endorphins in the bioassay. Such possible effect would give a false increase of endorphin-like activity when compared to the control group in table 1, which did not receive any drugs. The fact that the actual values found in the postoperative group were markedly lower than the controls, rules out the presence of any drug-related opiate-like activity in those samples. In addition, the extraction procedure used in this study is not efficient for the extraction and recovery of fentanyl and droperidol.

It is tempting to conclude from our results that there is a causal relationship between low endorphin levels and acute pain, as has been suggested by the effects of naloxone administration.[18] However, the effects of surgical stress on endorphin production and release are not known. In addition, these patients were exposed to a variety of drugs which may alter endorphin levels in CNS. Based on these considerations, one may interpret the exceedingly low values observed in our postoperative patients as the result of CNS endorphin depletion by factors other than pain itself.

The authors thank Dr. F. Rodriguez-López head of Anesthesiology in C.S.V. Arrixaca, Murcia, Spain for his help and Ms. Carol Chesler for her assistance in preparing the manuscript.

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