

Endorphin Activity in Cerebrospinal Fluid Prior to Elective Cesarean Section and in Early Puerperium

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ABSTRACT

Opioid activity in cerebrospinal fluid (CSF) was estimated by radioreceptor-assay (RRA) in samples obtained from ten women at term pregnancy and in the early puerperal period. The samples were fractionated on Sephadex[®] G-10 columns and two opioid receptor active fractions, FI and FII, were recovered.

Two pools of FII materials from pregnant and puerperal women, respectively, were further analyzed by electrophoresis and the concentrations of opioid activity were measured by radioreceptorassay.

There was a significant rise in receptor-assayed FII opioid activity in late pregnancy as well as in the early puerperal period compared to that of healthy, nonpregnant, nonpuerperal females. Pooled FII material obtained before delivery could be separated into two major components tentatively assigned the hexapeptide [Met]enkephalin-Lys⁶ and the heptapeptide [Met]enkephalin-Arg⁶-Phe⁷. These two opioid peptides both have their origin in the [Met]enkephalin precursor, proenkephalin A. In the puerperal period there was predominance of only one of these components.

INTRODUCTION

The known number of endogenous opioid peptides (endorphins) which may act as ligands to opiate receptors has increased rapidly in the past few years. By the use of molecular genetics, the amino acid sequences of three different peptide precursors to these peptides have been predicted. The first, proopiomelanocortin, is the precursor of β -endorphin (13). The other two are proenkephalin A (1, 6, 12) and proenkephalin B (10), precursors to enkephalins and dynorphins, respectively. Thus, there are three families of endorphins

which show affinity in varying degree to different types of opioid receptors.

Endogenous opioids have been reported to provide pain relief in labour. Thus Oyama et al. in 1980 (18) described complete analgesia in 14 women who had received 1 mg of β -endorphin by intrathecal injection in the early stage of labour. A gradual rise in the pain threshold for electric stimuli during the final week prior to parturition has also been registered in rat experiments (4). The pain threshold was rapidly normalized following delivery and it was also abolished in naltrexone treated pregnant animals. Prenatal naloxone also affected morphine sensitivity of the off-spring (7).

In the present investigation a screening of cerebrospinal fluid (CSF) substances with opioid receptor affinity was conducted in late pregnancy and in the puerperal period. CSF samples were obtained near the end of the third trimester before onset of labour and also during the early puerperal period in the same women. The aim of the study was to establish whether the amounts of endorphins in CSF are increased in the final stage of pregnancy and if such an increase could be related to any of the three opioid peptide precursors.

MATERIAL AND METHODS

The study included two groups of women.

- 1) One group was composed of 10 healthy pregnant women (mean age 30 years, range 22-37) who underwent elective cesarean section at term due to pelvic disproportion. The women had all volunteered for the study. There were no drugs given prior to delivery. The operations were performed during spinal anesthesia and prior to administration of the anesthetic drug, a 5 ml sample of CSF was removed through a 25 gauge needle. The cesarean sections were all performed between 10 and 12 am and the anesthetic used was 50-75 mg of Xylocain[®]. A second lumbar puncture was done one week post partum when another 5 ml of CSF was sampled.
- 2) The control group was composed of 16 nonpregnant, nonpuerperal healthy females who volunteered for the study. The mean age of this group was 26.1 years (range 18-35). Samples of 5 ml CSF were obtained from each woman with the same technique as for the women in group 1.

The investigation was approved by the Ethics Committee of the Medical Faculty.

The lumbar punctures were done at the level of L:3-L:4 with the woman in a

sitting position. The average collection time was 15 minutes. CSF samples were taken in plastic tubes, frozen and kept at -70°C until analyzed.

Biochemical methods

The standard peptides [Met]enkephalin and [Met]enkephalin-Arg⁶-Phe⁷ were obtained from Bachem, Bubendorf, Switzerland, whereas dynorphin₁₋₈ and [Met]enkephalin-Lys⁶ were supplied by Peninsula Laboratories, San Carlos, USA. Equipment for gel filtration (Sephadex[®] G-10) and the agarose (Agarose-C) used in the electrophoretic separation were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

The opioid activity in each sample was analyzed according to Terenius and Wahlström (20). In this method the opioid active material is separated into two major fractions called FI and FII, by chromatography on Sephadex G-10 columns. These fractions are further tested for receptor-binding affinity in a radioreceptorassay (RRA), which uses synaptic plasma membranes from rat brain excluding cerebellum with ³H-labelled dihydromorphine as a competing radioligand. Each assay included a calibration curve with [Met]enkephalin, and the binding activity of the tested fractions was expressed in [Met]enkephalin equivalents. The biochemical characteristics of these fractions have been studied extensively in this laboratory (15, 16, 17). The contents of fraction I include hydrophilic peptides with eight or more amino acids such as dynorphin A (dynorphin₁₋₁₇) and its shorter fragments (i.e. dynorphin₁₋₈) while fraction II contains enkephalin peptides with six to eight amino acids including [Met]enkephalin-Lys⁶ and [Met]enkephalin-Arg⁶-Phe⁷.

Column electrophoresis in agarose suspension of pooled FII material was performed according to Nyberg and Terenius (16). For this procedure, the freeze-dried FII materials from the ten pregnant women were combined into two pools, one consisting of material collected in term pregnancy before cesarean section and one consisting of FII material from samples collected in the early puerperal period. The duration of each electrophoresis was five hours at a current of 10 mA and a voltage of 1,000 V. Fractions of 0.4 ml were then suctioned from the column and separated from agarose by the use of a centrifuge. The distribution of the endorphin activity was monitored by radioreceptor-assay. Calibration of the column had been performed earlier with samples of known enkephalin- and dynorphin-related peptides.

RESULTS

The CSF levels of FI and FII in the cesarean section group and in the control group are shown in Figs. 1 and 2. In these patients, no general increase was found in FI either at the time of cesarean section or in the early puerperium in comparison with the control group. FII, on the other hand, was significantly increased both before cesarean section and in the early puerperium compared to the control group ($p < 0.01$ and $p < 0.01$, respectively, Student's t-test on independent samples; $p < 0.01$ and $p < 0.01$, Mood's median test). There was no significant change in FII levels in the early puerperium compared to those in late pregnancy (t-test on paired samples; Mood's median test). Two women (no. 1 and no. 7) had very high FII levels at the time of cesarean section. Following delivery this fraction decreased. In one of the women (no. 1) a similar pattern was found for FI while for the other woman FI was not elevated.

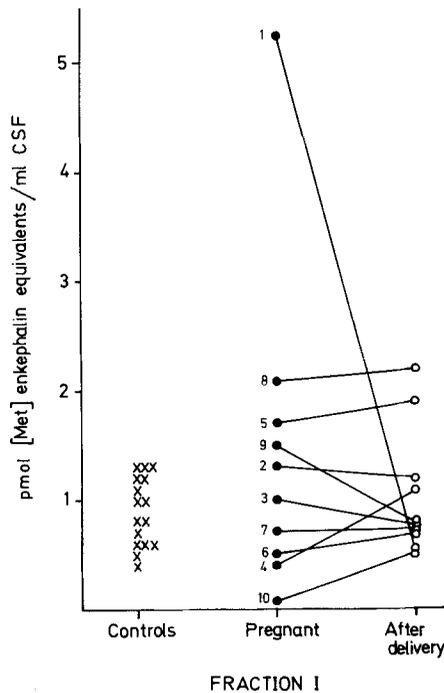


Fig. 1 CSF levels of Fraction I opioid peptides in 10 women prior to cesarean section (\bullet), in early puerperium (\circ), and in 16 healthy female controls (\times).

After column electrophoresis of the pooled FII material from samples in late pregnancy, the opioid activity could be separated mainly into two components. Fig. 3a shows the levels of these components in the group of ten women prior to cesarean section. In the puerperal period there was a predominance of only one of these components (Fig. 3b).

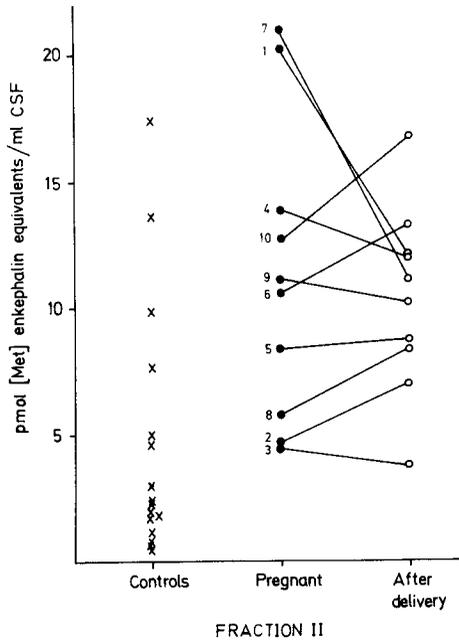


Fig. 2 CSF levels of Fraction II opioid peptides in 10 women prior to cesarean section (●), in early puerperium (o), and in 16 healthy female controls (x).

DISCUSSION

There are two principal methods to measure opioid peptides in body fluids: by radioreceptorassay (RRA) and radioimmunoassay (RIA). RIA is the most commonly used method when analyzing endorphins in body fluids. The major advantage of the RIA is that it will give information about individual opioid peptides while RRA determinations are based on functional opioid activity. Though RRA does not distinguish between individual endorphins it will, however, give a more complete information about the total amount of endorphin

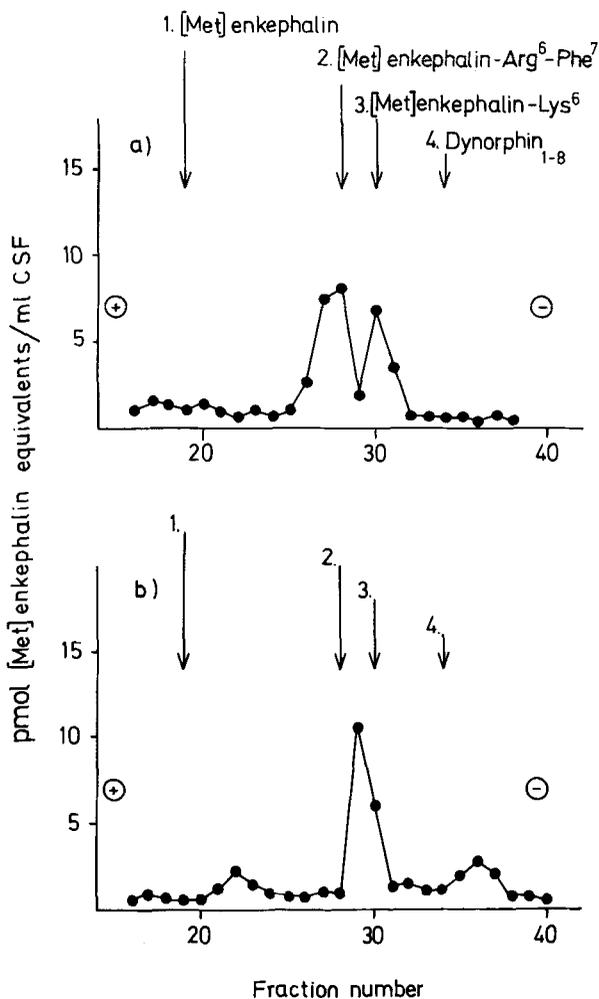


Fig. 3 Column electrophoresis (pH 2.7) in agarose suspension of pooled CSF Fraction II opioid peptides from 10 women: a) in term pregnancy prior to elective cesarean section and b) one week following delivery.

Fractions were isolated and subjected to radioreceptorassay. For comparison, runs of some known opioid peptides are also shown in the figure.

activity. It has earlier been shown in this laboratory by Nyberg and Terenius (15) that classical endorphins such as enkephalin, β -endorphin and dynorphin contribute less than 10 per cent of the total receptor activity in CSF.

The activity of the endocrine system during pregnancy and in labour has been studied by several investigators. The results reported on β -endorphin and β -endorphin-like peptides in maternal plasma during pregnancy and delivery are

rather conflicting. Goland et al. (5) found no difference in β -endorphin plasma concentrations in either the first, second or third trimester in comparison with values in nonpregnant women. Genazzani et al. (3) found progressively increased β -endorphin plasma levels from the 27th week of gestation and the highest concentration at term. Newnham et al. (14) reported a pattern similar to that of Genazzani but also a progressive rise even for β -lipotrophin. Newnham's group also reported unchanged levels of maternal plasma [Met]enkephalin during pregnancy. On the other hand, Hoffman et al. (8) found significantly lower plasma levels of β -endorphin in each trimester of gestation than in the levels of nonpregnant control subjects.

Much less is known about opioid peptide secretion within the CNS and reports on endorphins in CSF during pregnancy are rare. Datta et al. (2) found no differences in CSF levels of immunoreactive β -endorphin between nonpregnant women and women at term prior to elective cesarean section. In fact, the CSF levels showed no elevation at term after active labour for 6 to 8 hours.

β -endorphin is synthesized both from the anterior lobe of the pituitary gland and from the brain. Schlachter et al. (19) found significant amounts of this peptide in CSF, but not in plasma, five days after complete hypophysectomy in 13 patients. No correlation between the concentrations of β -endorphin in plasma and in CSF has been found (2, 19).

The present study provides evidence that there is an increased amount of substances in CSF with opioid receptor affinity in late pregnancy as well as in early puerperium compared to those found in nonpregnant, nonpuerperal women. At the lumbar level we found that most of opioid receptor-active material in FII could be traced to proenkephalin A which is the [Met]enkephalin precursor. Since enkephalins seem to be the main opioid peptides in the spinal cord (9) this is not unexpected. There were, however, no signs of increased levels of the pentapeptides [Met]enkephalin or [Leu]enkephalin in the CSF samples. Instead, electrophoresis gave evidence of other forms of enkephalin-containing polypeptides (ECPs), tentatively assigned the hexapeptide [Met]enkephalin-Lys⁶ and the heptapeptide [Met]enkephalin-Arg⁶-Phe⁷. Kofinas et al. (11) found that vaginally delivered women had significantly higher plasma β -endorphin levels than those women delivered by cesarean section. Thus, stress caused by cesarean section does not seem to elevate circulating amounts of this opioid peptide. Our finding that there is a moderate FII increase in late pregnancy before elective cesarean section is most likely not only an effect of stress in connection with the operation.

The role of endorphins in the physiology of pregnancy is at present unclear. The differences in CSF levels of some endorphins before and after delivery

found in this study indicate that endogenous opioid peptides may play a role in these events also in humans. Further studies are needed in order to elucidate the importance of the different opioid peptides for the delivery and the adaptation of labour pains in humans. Such studies are under progress.

ACKNOWLEDGEMENTS

The authors are grateful to Ms Inga Hansson for skillful technical assistance. This work was supported by Syskonen Svensson's Fond and the Swedish Medical Research Council, grant No. 5095 (L. Lindström).

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