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Effects of Epidural Anesthesia during Labor on Maternal Plasma Beta-Endorphin Levels

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Plasma beta-endorphin (β -EP) was measured in 48 women. Twenty-three were in labor. In 13 of the 23 patients in labor, β -EP was determined prior to and after complete onset of epidural anesthesia, and in 10 women, who served as controls, prior to and after injection of saline into the epidural space as part of the loss of resistance technique, but before injection of the local anesthetic. Venous blood also was obtained for plasma β -EP determinations from 10 healthy non-pregnant women and from 15 patients scheduled for elective repeat cesarean section and who were not in labor. Human β -EP was determined by radioimmunoassay following silicic acid extraction of plasma samples and separation of the β -EP fraction by gel chromatography. In the 10 non-pregnant volunteers, plasma β -EP averaged 11.3 ± 1.5 fmol/ml (mean \pm SE) as compared with 43.7 ± 6.5 fmol/ml observed in the 15 women with term pregnancies who were not in labor ($P < 0.005$). In the 13 patients in labor who underwent epidural anesthesia, plasma β -EP concentrations decreased ($P < 0.005$) from 54.5 ± 9.0 to 28.2 ± 3.5 fmol/ml, whereas there was no significant change in plasma β -EP levels in the 10 controls who averaged 64 ± 20.5 and 55.8 ± 13.6 fmol/ml prior to and following saline injection. These data confirm that plasma β -EP levels are significantly higher in women with term

pregnancies in labor than in non-pregnant women and also demonstrate that epidural anesthesia during labor is accompanied by a significant decrease in maternal plasma β -EP concentrations. (Key words: Anesthesia: obstetrics. Polypeptides: beta-endorphin. Pregnancy: labor.)

BETA-ENDORPHIN (β -EP) is a peptide hormone that consists of the 31 C-terminal amino acids of the 91 amino acid peptide β -lipotropin from which β -EP is derived by selective cleavage.¹ Recent evidence suggests that β -EP as well as other endorphins (endogenous peptides which bind specifically to opiate receptors in the CNS) are neurotransmitters of specific neural systems that mediate the integration of sensory information pertaining to pain perception and emotional behavior.² Endorphins have been shown to be involved in CNS mechanisms that affect pain tolerance.^{3,4} Furthermore, peripheral blood levels of β -EP and related peptides were found to be increased in response to stressful conditions associated with hypoxia and acidosis in human adults⁵ and newborns.^{6,7} Because labor is a stressful condition, this study was undertaken to examine the effect of epidural anesthesia during labor upon maternal plasma β -EP concentrations.

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Materials and Methods

SUBJECTS

Antecubital venous blood was collected from 10 healthy female volunteers who were not pregnant, from 15 patients with uncomplicated term pregnancies who were scheduled to undergo elective repeat cesarean section prior to the onset of labor, and from 23 patients with uncomplicated term pregnancies who were in active labor and elected to undergo epidural anesthesia

during labor and delivery. Informed consent was obtained from all subjects who volunteered to participate in this study which was approved by the Human Research Committee of the Los Angeles County/University of Southern California Medical Center. Epidural catheters were placed in all of the latter group of 23 patients in labor at the L3–L4 interspace using the loss of resistance technique with saline. All patients received 500 ml of 5% dextrose in lactated Ringer's solution and were in supine position with left uterine displacement. These 23 patients were divided randomly into two groups: blood was collected from the first group prior to and after the injection of saline and a local anesthetic and the onset of epidural anesthesia (approximately 30 to 40 min apart), and from the second (control) group prior to and 30 to 40 min following the injection of saline into the epidural space, but before the injection of the anesthetic. Either 0.5% bupivacaine or 2% 2-chloroprocaine was used as local anesthetic. All blood samples were aspirated into plastic syringes and transferred immediately into chilled EDTA tubes, mixed with the EDTA, placed in a bucket with crushed ice, and centrifuged within 15 min at 4° C to obtain the plasma. The latter was stored in glass tubes at –20° C until it was analyzed for β -EP.

ASSAY

Human β -EP was measured by radioimmunoassay (RIA) as described previously.⁷ In brief, β -EP, together with other peptides, is extracted from plasma by the silicic acid method of Krieger *et al.*⁸ and Wilkes *et al.*⁹ using siliconized glassware throughout the procedure except for the RIA proper. Prior to the extraction, approximately 2,000 cpm of ¹²⁵I-labeled β -EP (about 1.7 fmol**) is added to all plasma samples in order to monitor recovery and correct for procedural losses in every assay. The peptides are eluted from the silicic acid with a mixture of acetone and 0.1 N HCl (4:6/v:v). The eluate is evaporated under a gentle stream of dry air at room temperature. The residue is dissolved in phosphate buffer saline (PBS: 0.02 M sodium phosphate buffer, pH = 7.4, 0.15 M NaCl, 10 mM EDTA, 0.1% gelatin, 0.1% sodium azide) and subjected to gel chromatography on a 180 × 6 mm Sephadex® G-50 column which separates the β -EP fraction from the preceding beta-lipotropin (β -LPH) fraction. Tests in which either iodinated or large amounts of cold β -LPH were added to plasma samples with low endogenous β -EP and β -LPH concentrations revealed that the added β -LPH did not result in a detectable increase in the β -EP fraction indicating separation of the β -EP from the β -LPH frac-

tion. Approximately 75% of the β -EP subjected to gel chromatography is recovered in the β -EP fraction as collected in our procedure in a total volume of 1.4 ml. Duplicate 0.4 ml-aliqouts of the β -EP fraction are then subjected to RIA that utilizes ¹²⁵I-labeled human β -EP prepared by the chloramine-T method as described by Hunter and Greenwood.¹⁰ Our anti- β -EP serum was raised in rabbits who were immunized with human β -EP (Beckman Instruments, Inc., Palo Alto, California) that had been coupled to bovine serum albumin by the bis-diazotized benzidine method¹¹ as described by Wilkes *et al.*⁹ The antiserum used in this RIA cross-reacts essentially 100% on a mole per mole basis with β -LPH but does not cross-react with other peptides such as human β -MSH, human ACTH (1-39), ACTH (1-24), methionine enkephalin, or leucine enkephalin. The RIA utilizes the same buffer that is used for gel chromatography, human β -EP as the standard, and a second antibody plus polyethylene glycol to separate bound and unbound ¹²⁵I- β -EP. Logit transformation is employed to construct standard curves and to calculate the β -EP concentrations in the unknown plasma samples.¹² The sensitivity of the assay is approximately 7 fmol/ml if a 5-ml plasma aliquot is analyzed. Intra- and interassay coefficients of variation are 6.8 and 11%, respectively, and the recovery of β -EP added to plasma aliquots is virtually complete (96%). Mean values were compared using the one-way analysis of variance. When the overall comparison was significant, a two-tailed Student's *t* test was applied. A *P* < 0.05 was considered significant statistically.

Results

The means of the volunteers' ages, weights, heights, parities, gestational ages and weights of the newborns determined for the nonpregnant controls, the patients awaiting elective repeat cesarean section, the patients in labor who underwent epidural anesthesia, and those into whose epidural space only saline was injected (Groups 1 through 4, respectively) are listed in table 1. These data do not reveal significant differences with three unimportant exceptions: 1) the non-pregnant controls, on the average, were older than the patients of all other groups; 2) the patients scheduled for elective cesarean section were shorter than Group 1 and 3 subjects; and 3) the latter had delivered more children than Group 3 volunteers.

As shown in figure 1, the mean (\pm SEM) plasma β -EP concentration found in the non-pregnant controls (11.3 ± 1.5 fmol/ml) was significantly (*P* < 0.005) lower than that of the patients with term pregnancies awaiting elective repeat cesarean section prior to labor (43.7 ± 6.5 fmol/ml), those in labor who were to receive epi-

** One fmol of β -EP is equal to 3.465 pg.

TABLE 1. Comparison of Groups of Volunteers Studied (Means \pm SE)

	1 Non-pregnant Controls (n = 10)	2 Pregnant Not in Labor (n = 15)	3 In Labor Epidural Anesthesia (n = 13)	4 In Labor No Epidural Anesthesia (n = 10)
Age (yr)	31.0 \pm 2.2*	25.9 \pm 0.9	21.7 \pm 1.1	25.0 \pm 1.9
Weight (kg)	62.7 \pm 6.0	74.7 \pm 3.5	71.8 \pm 2.8	68.6 \pm 1.7
Height (cm)	160.0 \pm 1.9	154.3 \pm 1.1†	160.0 \pm 2.8	157.5 \pm 1.4
Parity	1.7 \pm 0.4	2.3 \pm 0.2‡	1.4 \pm 0.2	2.3 \pm 0.7
Gestational age (wk)		40.1 \pm 0.8	39.7 \pm 0.7	39.4 \pm 0.5
Infant weight (kg)		3.459 \pm 0.089	3.467 \pm 0.151	3.353 \pm 0.078

* Different from Groups 2, 3, and 4 ($P < 0.025$).

† Different from Groups 1 and 3 ($P < 0.02$).

‡ Different from Group 3 ($P < 0.01$).

dural anesthesia (54.5 ± 9.0 fmol/ml), and those in labor who served as controls by initially receiving only saline (64 ± 20.5 fmol/ml). There was no significant difference between the peripheral plasma β -EP levels of Group 2, 3, and 4 patients, however. Following injection of the local anesthetic into the epidural catheters, a significant ($P < 0.005$) decrease in maternal plasma β -EP levels to 28.2 ± 3.5 fmol/ml was observed. Saline injection into the epidural space alone did not alter maternal plasma β -EP concentrations significantly: Group 4 patients still averaged 55.8 ± 13.6 fmol/ml 30 to 40 min after the saline was injected as part of the loss of resistance technique. Although epidural anesthesia caused a decrease in maternal plasma β -EP concentrations, the resulting levels were still significantly (P

< 0.005) higher than those of the non-pregnant controls.

Discussion

The identification of specific opiate receptors in the CNS in 1971 to 1973 suggested that the function of these receptors was to combine with endogenous opioid substances. A deliberate search for such ligands resulted in 1975 in the discovery of the endorphins, peptides with opiate-like pharmacologic activity.^{13,14} These peptides are present throughout the brain and spinal cord with varying concentrations in different regions. Growing evidence from animal and human studies supports the hypothesis that the endorphin system plays a role in pain perception,^{15,16} in the regulation of biologic re-

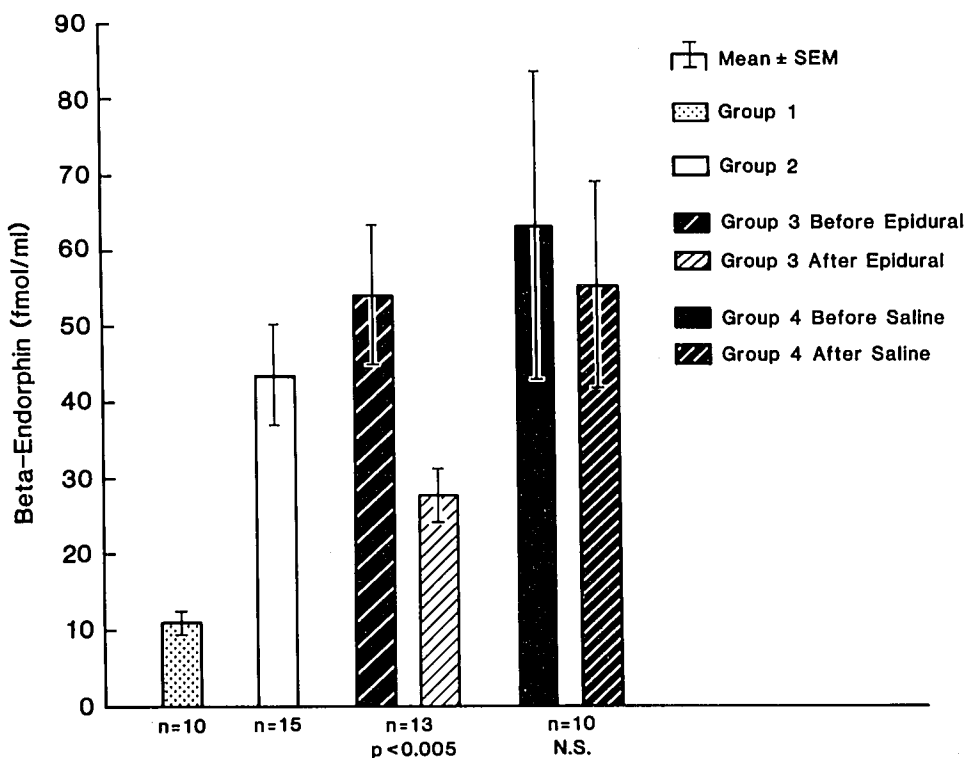


FIG. 1. Beta-endorphin concentrations in antecubital venous plasma obtained from 10 non-pregnant women ("non-pregnant," Group 1), from 15 patients prior to elective repeat cesarean section who were not in labor ("pregnant, not in labor," Group 2), from 13 women with uncomplicated term pregnancies in active labor prior to and 30 to 40 min after epidural anesthesia ("labor before epidural/labor after epidural," Group 3) and from 10 patients with uncomplicated term pregnancies in active labor prior to placement of an epidural catheter and 30 to 40 min after saline injection into the catheter ("labor before saline/labor after saline," Group 4).

sponse to stress,¹⁷ and possibly in the mediation of anesthetic action.¹⁸ Because pregnancy, labor, and parturition are states that require adaptation to stress, considerable attention has been focused on the possible involvement of endorphins in the physiologic changes that are associated with pregnancy. It has been shown that the pain threshold increases in rats during pregnancy and that this increase is abolished by the administration of the narcotic antagonist naltrexone,¹⁹ suggesting that the endorphin system modulates the responsiveness to aversive stimuli during pregnancy. Akil *et al.*²⁰ reported an increase in maternal plasma β -EP levels in a limited number of women between six months' gestation and the onset of labor as compared with non-pregnant controls, whereas Goland and collaborators²¹ did not find elevated maternal plasma β -EP levels prior to labor. However, the latter authors,²¹ as well as others,^{22,23} reported a rise in maternal plasma β -EP levels during active labor. Our data confirm the finding that maternal plasma β -EP concentrations are elevated significantly during labor. Our observation that maternal plasma β -EP levels also are elevated in patients with uncomplicated term pregnancies who are not in labor but scheduled to undergo elective repeat cesarean section must be interpreted with caution. The emotional stress that is associated with the anticipation of major surgery in the operating room without any premedicant drugs being given may have caused or contributed to the increase in circulating β -EP levels in this group of patients, and further studies are necessary to determine whether or not maternal plasma β -EP levels are elevated in pregnant women at term prior to the onset of labor. If there was an increase in circulating β -EP levels in pregnancy prior to the onset of labor, this would explain the decrease in anesthetic requirements for pregnant women that was reported by Palahniuk *et al.*²⁴ and thought to result from the increase in progesterone that is known for its sedative effects.

Our data demonstrate a significant reduction in maternal plasma β -EP levels in response to epidural anesthesia during active labor. These findings are in agreement with those reported by Thomas *et al.*²⁵ who found that meperidine and extradural blocks were associated with lower levels of β -EP when given to patients in labor. The physiologic significance of increased maternal plasma β -EP levels during labor and their partial suppression by epidural anesthesia remains unknown. Recently, a variety of evidence has shown that the endorphin system plays an important role in the biologic response to stress.^{17,26} Peripheral levels of β -EP have been found to be an indicator of various stressful conditions including labor,²¹⁻²³ hypoxia and acidosis in human adults⁵ and newborns,^{6,7} and surgical stress.²⁷ It is tempting to speculate that the fall in circulating β -EP,

which has a short half-life,²⁸ is the result of decreased pituitary β -EP secretion in response to the alleviation of labor pain. One cannot attribute central analgesic effects to the peripheral plasma β -EP levels measured during labor because even with plasma β -EP concentrations higher than those found by us and others²¹⁻²³ during labor, β -EP does not appear to cross the blood-brain barrier to effect analgesia in humans.²⁹ It is conceivable, however, that the release of pituitary β -EP into the peripheral circulation may occur concomitantly with alterations in the release of β -EP and/or other opioid peptides within certain regions of the CNS in response to the pain that is associated with labor and delivery. Such CNS analgesic activity of β -EP has been demonstrated in humans by intraventricular (third ventricle)³⁰ and intrathecal (lumbar puncture)^{31,32} administration, and it also has been shown that β -EP produces more potent analgesia in animals than morphine when compared on a molar basis.³³⁻³⁷

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