Anesthesiology
57:468–472, 1982

Effects of Fentanyl on the Response of Plasma Beta-Endorphin Immunoreactivity to Surgery

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Beta-endorphin appears to play a definite role in the biologic response to stress and in the endogenous mechanism of pain perception. Opiates exogenously administered during surgery decrease or even suppress the activation of "stress hormones" such as ACTH and cortisol. In the present study, the authors tried to assess the effects of fentanyl administration on plasma beta-endorphin immunoreactivity PBE(ir) during surgical stress.

In one group of nine patients, a standard enflurane-based general anesthetic technique without opiates was used for a staging laparotomy. A second group of ten patients undergoing the same type of surgery received fentanyl (10–20 μg/kg) as the primary anesthetic drug. In both groups, multiple blood samples were collected prior to, during, and after surgery, following the same time protocol. PBE(ir), plasma cortisol and, in five patients, plasma ACTH were determined by radioimmunoassay.

There was no significant change in PBE(ir) in either group after anesthetic induction. Unlike the enflurane group, the fentanyl group did not demonstrate any significant increase from baseline in PBE(ir) during surgery. There was a significant group difference between enflurane and fentanyl in PBE(ir) levels for both "early" and "late" surgery values, but not for the "awake" values (recovery period) where both groups had elevated PBE(ir) levels. Plasma cortisol and plasma ACTH changes followed a trend similar to those of PBE(ir).

The suppression of both cortisol and PBE(ir) responses during surgery after administration of fentanyl provides further evidence for the involvement of the endorphin system in the stress response and for its physiologic association with the hypothalamo pituitary axis. (Key words: Anesthetics, intravenous: fentanyl. Anesthetics, volatile: enflurane. Hormones: ACTH, cortisol. Polypeptides: endorphins.)

THERE IS GROWING EVIDENCE that the biologic response to stress involves the endorphin system. Increases in plasma beta-endorphin immunoreactivity [PBE(ir)] have been found in animals subjected to a variety of stresses.[1–3] Clinical studies have demonstrated that surgical stress also is associated with significant increases in PBE(ir)[4,5] and that the activation of the endorphin system is linked to activation of the hypothalamic-pituitary-adrenal (HPA) axis as reflected by a parallel variation in ACTH and cortisol.

The administration of high to medium doses of opiates has been shown to reduce cardiovascular, metabolic, and hormonal manifestations of surgical stress in humans.[6–8] In the present study, we have attempted to assess the effects of the intravenous administration of fentanyl on PBE(ir) during surgical stress and to determine the possible interaction between the opiate and the opioid peptide.

Materials and Methods

PATIENT SELECTION

Following institutional approval, nineteen patients (two women and 17 men; age range: 20–70 years) were selected, gave their informed consent, and underwent laparotomies as part of National Cancer Institute treatment protocols. They were free of cardiovascular, respiratory, renal, or neurologic abnormalities. Their preoperative medical assessment was remarkable only for the existence of regional malignancy. They received no medication for two weeks prior to surgery.

ANESTHETIC TECHNIQUE

Pentobarbital (1.5 mg/kg, im) was given for premedication. The anesthetic drugs consisted of thiopental (5–9 mg/kg) and succinylcholine (1–1.5 mg/kg) for induction, and nitrous oxide (66%) and pancuronium (0.1–0.15 mg/kg) for maintenance. In addition, one group (10 patients) received a medium dose of fentanyl (10–20 μg/kg), given iv 5 min before the surgical incision. The other group (nine patients) received enflurane as the primary maintenance anesthetic. All patients were intubated and had controlled normoventilation. The surgery lasted between 2 and 4 h.

BLOOD COLLECTION

Blood samples were collected from each patient prior to and 10 min after anesthetic induction (before the

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Received from the Clinical Center, National Institutes of Health, Bethesda, Maryland 20205. Accepted for publication May 19, 1982.

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0003-3022/82/1200/0468 $01.05

TABLE 1. Plasma Beta-Endorphin Immunoreactivity (pmol/l)

	Patients									
	1	2	3	4	5	6	7	8	9	10
Fentanyl group										
Pre-induction	15.4	<14	<14	18.9	<14	22.7	14.9	<14	18.2	22.8
Early surgery	<14	<14	<14	<14	<14	21.7	<14	19.3	<14	<14
Late surgery	36.4	<14	<14	15.1	101.5	21.8	15.3	42.7	<14	<14
Awake pre-morphine	30.1	<14	39.9	15.1	158	64.3	23	44.9	<14	34.1
Enflurane group										
Pre-induction	<14	<14	31	28.2	32.6	16.5	<14	<14	<14	
Early surgery	56.9	26.6	109.3	45.1	121.9	47.1	23.4	<14	49.1	
Late surgery	70.7	16.2	63.8	51	49	120	33.9	20.9	32.7	
Awake pre-morphine	24.4	75.8	—	73	35.3	215	40.9	23.4	—	

administration of either enflurane or fentanyl), 10 min after skin incision, at 30-min intervals during surgery until skin closure, and when awake in the Surgical Intensive Care Unit, prior to administration of morphine sulfate for postoperative analgesia.

ASSAYS

PBE(ir) and plasma cortisol were determined by radioimmunoassays (RIA) in all patients. In a group of five patients (three given fentanyl), plasma levels of ACTH were determined by RIA. Both PBE(ir) and cortisol radioimmunoassays used standard rabbit antibodies supplied by New England Nuclear. In human plasma, the beta-endorphin antiserum, raised against human beta-endorphin, shows less than 5% cross-reactivity with ACTH (M. Cohen, unpublished data), less than 50% cross-reactivity with beta-lipotropin, less than 0.01% with alpha-endorphin and alpha-MSH, and less than 0.004% with met- and leu-enkephalin (New England Nuclear data). Blood samples, collected in polypropylene tubes containing bacitracin (2 mg/ml) to inhibit proteolysis, and EGTA anticoagulant (20 mM) were placed immediately in ice and spun within 10 min at 3,000 rpm for 15 min at 4°C. The plasma obtained was frozen at −70°C and assayed within 3 weeks. Plasma samples from each patient were run on the same assay. Intra-assay variation for plasma cortisol was 6% and for beta-endorphin 3.5%, and interassay variations were 8% and 5%, respectively. For ACTH, the antiserum was provided by Immunological Nuclear Corporation. (Interassay variation: 20%; intra-assay variation: 3.6%). All assays were carried out using unextracted serum. All samples were coded and assigned blindly by non-laboratory personnel.

DATA ANALYSIS

Blood samples were grouped into four surgical time periods: pre-induction values, "early" surgery (first three samples taken after incision during the first 70 min), "late" surgery (subsequent half-hour samples until skin closure), and awake (prior to administration of analgesic). For cortisol values, statistical analysis was performed by Student's t test for paired samples to assess changes between surgical conditions, and t test for independent samples on changes (from pre-induction) to assess differences between the two groups (fentanyl and enflurane) for each surgical condition. A number of samples showed levels of PBE(ir) below the sensitivity of the assay (less than 14 pmol/l), especially after the injection of fentanyl. As a result, these data did not lend themselves to analysis by parametric statistics since group variances would have been biased. "Within patients-between conditions" analyses therefore were carried out using the non-parametric "signed rank test" for paired samples. For between-groups analysis, the non-parametric "Wilcoxon's rank sum test" for independent samples was used to evaluate changes. Finally, a correlation between PBE(ir) and cortisol was done using both parametric (correlation factor) and non-parametric (χ^2 test) methods.

Results

Since there were no significant differences between pre- and post-induction means for PBE(ir) and cortisol,†† pre-induction values were chosen arbitrarily as a baseline for both variables. Raw data for PBE(ir) are presented in table 1. In contrast to the enflurane anesthesia group in whom the early and late surgery and the awakening periods each were associated with significant increases from baseline in PBE(ir) ($P < 0.01$, $P < 0.01$, $P < 0.005$, respectively), the fentanyl group

†† Means and SEM: 1) beta-endorphin (pM), pre-induction, 16.9 ± 1.1, post-induction, 15.2 ± 1.1 (fentanyl group); pre-induction, 19.8 ± 2.7, post-induction, 21.1 ± 3.7 (enflurane group); 2) cortisol (mg/dl), pre-induction, 15.3 ± 3.2, post-induction, 12.7 ± 2.9 (fentanyl group); pre-induction: 20.7 ± 3.0, post-induction: 25.6 ± 3.9 (enflurane group).

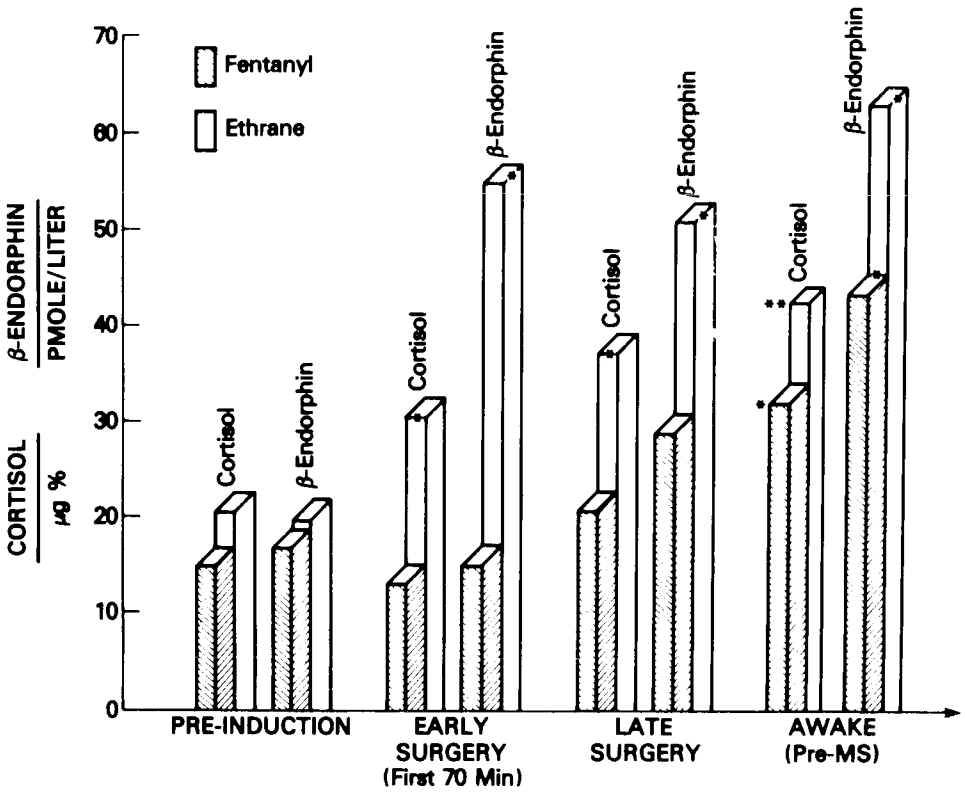

FIG. 1. Variations in cortisol and PBE(ir) in the fentanyl group, compared with the enflurane group.

showed significant PBE(ir) elevations in comparison to baseline only in the awake period ($P < 0.01$). This difference between the enflurane and the fentanyl group response was reflected by significant group differences in PBE(ir) values for both "early" and "late" surgery values ($P < 0.001$, $P < 0.05$, respectively), but not for the "awake" values (table 1).

Plasma cortisol showed changes during surgery which followed a similar trend to those of PBE(ir) (Fig. 1). In the enflurane group, early and late surgery and awakening conditions were associated with significant increases ($P < 0.01$, $P < 0.01$, $P < 0.001$, respectively), while in the fentanyl group only the awakening condition was associated with significant increases ($P < 0.01$): significant enflurane and fentanyl group differences were found for the "early" and "late" surgery conditions ($P < 0.001$, $P < 0.01$, respectively). Plasma ACTH showed a similar pattern in the five subjects in whom it was measured (table 2). A significant correlation across surgical and anesthetic conditions was found between cortisol and PBE(ir) (using 14 pmol/l as the lowest level of detectability) ($r = 0.48$, $P < 0.001$, $N = 71$). This relationship was confirmed by non-parametric analysis in which PBE(ir) and cortisol were divided into low and high values, with cut off levels of 16 pmol/l for PBE(ir) and 22 mg/dl for cortisol (table 3). Chi-square test for such a 2×2 contingency table was 26.5, df $= 1$, $P < 0.001$.

TABLE 2. ACTH Plasma Levels (pmol/l) in Five Patients

	Enflurane Group		Fentanyl Group		
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Pre-induction	13.5	12.2	23.7	<8.4	<8.4
Early surgery	54.3	19.7	16.4	<8.4	8.9
Late surgery	45.5	25.3	31.8	94.8	15.7
Awake	15.9	28.0	23.7	113.4	48.1

Discussion

We have already reported[5] that the endorphin system, as reflected by PBE(ir), is activated during surgery in patients receiving "non-narcotic" anesthesia. The responsiveness of the endorphin system to stress has been associated repeatedly with the activation of the HPA axis. Pain and stress evoke a decrease in hypothalamic endorphin activity,[2,3] augment the release of ACTH (and cortisol), prolactin, growth hormone, and ADH, as well as plasma BE(ir). All these hormonal changes have been reproduced in patients undergoing surgery with non-narcotic anesthesia.[8-11]

In the group of patients receiving fentanyl, a consistent and statistically significant suppression of PBE(ir) and cortisol was observed, an especially pronounced effect during the initial 70 minutes following the injec-

tion of the drug. This time interval of pharmacologic effects is consistent with the pharmacokinetic behavior of fentanyl at the dose range used in this study.[12] Large to moderate doses of opiates used in anesthesia are known to reduce the hormonal and metabolic responses to surgical stress, as well as to provide good cardiovascular stability. Morphine, 1 to 3 mg/kg,[13] and fentanyl, 50 to 100 μg/kg,[14] have been shown to have such effects. The lower doses of fentanyl used in our study, although not blocking the response completely, have consistently reduced the ACTH and cortisol response to surgery. A parallel suppression of the PBE(ir) response after administration of fentanyl provides further evidence for the involvement of the endorphin system in the response to stress and for its physiologic association with the HPA axis. Recently, a hypothalamic peptide which acts both as a corticotropin and a beta-endorphin-releasing factor, has been identified,[15] providing additional links between the two peptides.

As mentioned earlier, the beta-endorphin radioimmunoassay used in our study detects a composite of beta-endorphin and beta-lipotropin and plasma beta-endorphin represents only part of the PBE(ir) measured. However, analytical work using gel permeation chromotography (as in:16) suggests that increases in plasma beta-endorphin typically are paralleled by increases in plasma beta-lipotropin and that changes in PBE(ir) reflect consistent changes in plasma beta-endorphin. This might be predicted since beta-lipotropin is the precursor protein to beta-endorphin (beta-endorphin is the 61-91 portion of beta-lipotropin).

Plasma beta-endorphin may be released from the pituitary[1] or from other peripheral loci such as the pancreas.[17] There is also evidence that stress produces a mobilization of the CNS pool of beta-endorphin, with significant depression in its levels in both hypothalamus and periventricular beta-endorphinergic fibers.[3] This mobilization also is reflected by the high levels of beta-endorphin of hypothalamic origin which have been found in the hypophyseal portal blood.[18] In this light, it is possible that circulating beta-endorphin reflects CNS activation and thus may be a "marker" of CNS beta-endorphin release.

The administration of a potent opiate such as fentanyl, in doses sufficient to produce prominent analgesia, would be expected to occupy a fair amount of opiate receptors. This interaction between an exogenous opiate and the endogenous opioid system might be expected to produce alterations in PBE(ir). Our observation that fentanyl blunted surgical stress induced increases in PBE(ir), suggests that occupation of opiate receptors by exogenous opiates might interfere with or prevent the "stress mobilization" of endogenous ligands. An alternative suggestion could be a negative

TABLE 3. Numbers of Individual Plasma Values and their Correlation between PBE(ir) and Plasma Cortisol

	High PBE(ir) (>16 pmol/l)	Low PBE(ir) (<16 pmol/l)
High cortisol (>22 mg/dl)	24	10
Low cortisol (<22 mg/dl)	4	33

"opiate-endorphin" feedback mechanism, analogous to the "cortisol-CRF-ACTH" model, with site of relay perhaps in the hypothalamus. Additional support for such mechanisms may be derived from other clinical data. Dubois *et al.*[5] found that elevated levels of PBE(ir) and cortisol after awakening from surgery with non-narcotic anesthesia were reduced greatly by the postoperative administration of morphine. Cohen *et al.*[19] reported that surgically stimulated levels of PBE(ir) were inversely related to the total amount of morphine administered during the first 24 h postoperative for patient-assessed pain relief. Similarly, preoperative Fraction I CSF opioid activity has been reported to be inversely related to calculated mean CSF concentrations of meperidine which was self-administered by the patient for postoperative pain relief.[20]

The use of parenteral opiates in moderate to large doses to supplement general anesthesia is a relatively recent and increasingly utilized clinical approach[21] employed to reduce the cardiovascular and metabolic effects of surgical stress. The classic belief that stress is a vital protective mechanism is increasingly challenged.[22] The involvement of beta-endorphin and its interaction with exogenous opiates suggests that opiate anesthesia may modify the role of neuromodulator usually assigned to beta-endorphin in the organism's behavioral, visceral, and endocrine responses to stress. Activation or suppression of the endorphin system appears to be closely related to both the reasons for (*i.e.*, stress or pain) and the effects of (*i.e.*, pain relief) administering opiates during the perioperative period.

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