

## Anesthesia Does Not Increase Opioid Peptides in Cerebrospinal Fluid of Humans

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One theory of narcosis postulates that inhaled anesthetics produce general anesthesia by causing the release of endogenous opioid peptides. In the present study, however, the concentration of immunoreactive beta-endorphin-like material (eight patients) or leu-enkephalin (four patients) did not increase in cerebrospinal fluid of patients 5 min after induction of anesthesia with thiopental, 2-5 mg/kg and N<sub>2</sub>O 70%; after an additional 10 min, during which halothane was added; at 5, 15, and 60 min after surgical incision; or after 30 min in the recovery room. Therefore, any contribution of the endorphin system to the production of general anesthesia does not appear to require the release of beta-endorphin. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, intravenous: thiopental. Anesthetics, volatile: halothane. Brain: endorphins. Receptors: opiate. Theories of anesthesia.)

THE ROLE OF ENDOGENOUS OPIOID PEPTIDES (EOPs) in the production of general anesthesia with inhaled anesthetics is unclear. Finck *et al.*<sup>1</sup> suggested that anesthesia, in part, may be due to release of EOPs, since administration of naloxone increased the number of rats responding to a painful stimulus at a fixed anesthetic level. However, Harper *et al.*<sup>2</sup> were unable to confirm this observation, even at much higher doses of naloxone.<sup>2</sup>

Subsequently, Arndt and Freye<sup>3</sup> found that perfusion of the fourth cerebral ventricle with naloxone increased blood pressure and wakefulness in dogs anesthetized with halothane. They concluded that inhaled anesthetics probably exerted their action by provoking the release of substances having opiatelike actions. Similarly, in an editorial, Goldstein<sup>4</sup> also felt that general anesthetic agents may release enkephalin (or other endorphins) at opiate receptor sites at the pain pathways.

This controversy is not resolved easily. Many investigators have reported that the narcotic antagonists effectively antagonize a number of anesthetic actions,<sup>1,4-14</sup> whereas others reported no effectiveness.<sup>2,15-19</sup> To determine if general anesthesia induces release of EOPs we measured levels of immunoreactive beta-endorphin-like

material and leu-enkephalin in cerebrospinal fluid (CSF) before, during, and after anesthesia with thiopental, nitrous oxide, and halothane.

### Materials and Methods

We obtained approval from our local Committee on Human Research, and informed consent, to study eight patients undergoing electrical stimulation of periaqueductal gray matter with implanted electrodes for relief of chronic pain. As a part of the electrode implantation procedure, a cannula was placed in the cerebral lateral ventricle, and an Ommaya reservoir was placed subcutaneously for diagnosis and treatment of intraventricular hemorrhage or infection. The reservoir could be tapped percutaneously to obtain samples of ventricular CSF. Our studies were conducted during subsequent permanent implantation of the electrode, which was used with a radio frequency receiver for transcutaneous stimulation.

No narcotic analgetic or electrode stimulation was used by any patient in the 24 hours preceding surgery, and patients received no premedicant drugs. Immediately before anesthesia, the control sample (2 ml) of CSF was obtained. A second sample was obtained 5 min after induction of anesthesia with thiopental 2-5 mg/kg iv, which was supplemented by inhalation of 70% N<sub>2</sub>O and 30% O<sub>2</sub> via face mask. Halothane then was given to produce anesthesia to a depth sufficient for endotracheal intubation. After 10 additional minutes of nitrous-oxide-halothane anesthesia and just before surgical incision, the third sample of CSF was obtained. Additional samples were drawn 5, 15, and 60 min after incision and during nitrous-oxide-halothane anesthesia. The sixth sample was obtained after 30 min in the recovery room. For three patients, *plasma* samples were obtained concomitantly and were analyzed for immunoreactive beta-endorphin-like material.

To halt possible peptide degradation, tubes containing ventricular CSF were immersed immediately in boiling water for 10 min and then frozen. Radioimmunoassays for immunoreactive beta-endorphin-like material (eight patients) were performed as described by Guillemin *et al.*<sup>20</sup> The rabbit antiserum used in this assay quantitatively measures beta-endorphin. The usable range of the assay is from 50 pg to 5 ng beta-endorphin. Sensitivity of the assay is approximately 50 pg, and half maximal displacement is usually obtained at 200-300 pg. Radioimmu-

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TABLE 1. Amount of Immunoreactive Beta-endorphin-like Materials in Plasma and Ventricular Cerebrospinal Fluid (CSF)

Times of Obtaining Samples of CSF or Plasma	Immunoreactive Beta-endorphin-like Material		
	n	In CSF (pcg/ml)*	In Plasma (pcg/ml)†
Before anesthesia (control)	8	281 ± 42	940, —, 490
Thiopental-N <sub>2</sub> O anesthesia 5 min after induction	8	370 ± 105	250, 1,970, 300
Addition of halothane	7	290 ± 60	710, 1,480, —
5 min after incision	7	259 ± 45	730, —, 530
15 min after incision	8	251 ± 50	—, 1,600, 810
60 min after incision	7	260 ± 35	860, 1,390, —
After 30 min in recovery room	6	228 ± 41	760, 1,780, —

\* Values are means ± SE.

† Individual values for three patients.

noassay for leu-enkephalin (four patients only) was performed as described by Rossier *et al.*<sup>21</sup> Half maximal displacement with this assay is obtained at approximately 16 pg of leu-enkephalin, and the sensitivity is approximately 1 pg.

Statistical analysis of the data was done using repeated measures analysis of variance.

### Results

No significant changes were seen under any conditions of the experimental protocol (table 1). Examination of CSF (four patients only) for leu-enkephalin failed to demonstrate detectable levels of this peptide. Changes in immunoreactive beta-endorphin-like material in CSF did not correlate with changes in *plasma* levels.

### Discussion

For all patients, immunoreactive beta-endorphin-like material did not increase significantly in CSF during anesthesia with or without neurosurgery (table 1). Also, leu-enkephalin was not detectable in CSF. Therefore, the production of EOPs does not appear to contribute to the anesthetic state produced by thiopental, nitrous oxide, or halothane.

A possible criticism of our protocol might be that the level of immunoreactive beta-endorphin-like material in CSF might not accurately reflect actual brain concentration because of the time lag between release of peptide from the brain substance and its transport to the sampling site. However, at no time did concentrations increase significantly after surgical incision; *i.e.*, if there is release undetected by our method, the time lag would have to exceed 1.5–2 h. Such a delay has not been seen in our studies of release of EOP following stimulation of human periaqueductal gray matter for pain relief.<sup>22</sup> The method

of sampling in that study (Ommaya reservoir) was the same as in the present study, and increases in EOPs were found 15–30 min after stimulation.

It is important to recognize that the antisera used in the beta-endorphin assay, although measuring quantitatively beta-endorphin, also recognizes beta-lipotropin and the pre-pro hormone pro-opiomelanocortin. The recognition of these two molecules and their possible confusion with beta-endorphin may be dismissed by noting that the pro-opiomelanocortin primarily is metabolized by hypothalamic neurons to beta-endorphin before it reaches the CSF. Beta-lipotropin is a somewhat different story in that, although it may be detected falsely by this assay, its presence there falsely would increase the value of beta-endorphin, and, since beta-lipotropin has no analgetic activity, it can be viewed as a constant but unimportant aspect of our measurement.

In addition, beta-lipotropin, of course, is not found stored in those neurons nearest to where we are withdrawing our samples. Although no data are available on human CSF, to the best of our knowledge, it has been demonstrated in rats that approximately 65% of the immunoactivity demonstrated in this assay is due to beta-endorphin rather than to the two precursors. This issue is of minimal concern in any case, because we failed to find an increase in activity.

The rabbit antiserum used in this assay against beta-endorphin binds neither enkephalins nor other endorphins. Similarly, the antiserum used in the leu-enkephalin radioimmunoassay showed no cross-reactivity with endorphins.

Beta-endorphin clearly can produce analgesia in both animals<sup>23</sup> and humans<sup>24</sup> when administered intraventricularly in doses of 100–400 µg, and in humans the intensity and duration of analgesia are dose-related. Also, we demonstrated earlier that immunoreactive beta-endorphin-like material increased twofold to sevenfold after stimulation of the periaqueductal gray matter, an area rich in fibers that are able to react to beta-endorphin.<sup>22</sup> The immunoreactive beta-endorphin-like material observed in samples of CSF from the third ventricle (same method and site of sampling as used in our study) probably originated from nerve fibers located in the anterior hypothalamus and produced analgesia after reaching the third ventricle by binding with the many opiate mu receptors in periaqueductal gray matter. Thus, release of beta-endorphin probably plays an important role in certain types of analgesia, but not in that associated with general anesthesia.

Our data, of course, do not exclude other means by which the analgesia component of general anesthesia may be produced by the endorphin systems, such as a direct action on EOP receptors, sensitization of EOP receptors, or facilitation of EOP action, as suggested by Yaksh and Howe.<sup>25</sup> However, the report by Shingu *et al.*<sup>26</sup> suggests that general anesthetics (nitrous oxide, thiamylal, halo-

thane, ether, enflurane) do not increase the sensitivity of nociceptive neural mechanisms or facilitate EOP action in the cat. These investigators found that fentanyl, 30 µg/kg iv depressed both spontaneous and bradykinin-(intraarterial) induced multiunit activity in the lateral funiculus of the spinal cord and that this depression was *antagonized completely* by naloxone, 0.1 mg/kg. This dose is one-tenth to one-hundredth that used by most investigators who have found that naloxone antagonizes the effect of general anesthetics (e.g., Finck *et al.*<sup>1</sup>). Such high doses of naloxone may produce pharmacologic effects that have nothing to do with the endorphin receptors.<sup>2</sup> Shingu *et al.*<sup>26</sup> also found that nitrous oxide, thiamylal, halothane, and ether (but not enflurane) depressed bradykinin-induced firing in the lateral funiculus but that this effect was not antagonized by naloxone, 0.1–2.0 mg/kg. Nitrous oxide enhanced spontaneous firing, while the other anesthetics produced varying degrees of depression. Thus, our data and that of Shingu *et al.* do not support the hypothesis that anesthetics act in some fashion by increasing release of, or sensitivity to, EOPs.

The results of several groups indicate that mechanisms other than EOP systems may produce perioperative analgesia. First, analgesia produced by electrical stimulation of the brain is reversed only partially by naloxone.<sup>27,28</sup> Tolerance to analgesia produced in this way, and cross-tolerance to morphine, are also incomplete.<sup>29</sup> Furthermore, in humans, naloxone will reverse analgesia produced by acupuncture but not hypnosis.<sup>30</sup> Finally, only certain frequencies of transcutaneous electrical stimulation produce analgesia.<sup>31</sup> How these other mechanisms might be affected by general anesthetics remains to be determined.

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