Enkephalins and endorphins: The endogenous opiates

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The discovery of the opiate receptors in the central nervous system of man and subsequent identification of endogenous opiate-like ligands (enkephalins and endorphins) has provided a model for the analgesia, euphoria and addiction produced by the narcotics. In this article, the author reviews the background, biosynthesis and degradation, distribution, nociceptive transmission and analgesic effects of these opioid peptides.

Anesthesia is a clinical orchestration of analgesia, amnesia, hypnosis and muscle relaxation. Of these components, analgesia is of concern to the anesthesiologist in all phases of surgery: preoperatively, intraoperatively and postoperatively.

Although morphine is an effective drug for the relief of pain, its effects are broader than just relief. Hence, chemists have worked to create analgesics which are devoid of morphine's side effects, which include respiratory depression, decreased gastrointestinal motility, mood alteration, increased/decreased levels of specific hormones, tolerance and duration.

A central issue in the search for the "perfect" analgesic was to determine why and how morphine should work in the first place. Why should a plant alkaloid have a specific binding site in the central nervous system of man? Researchers reasoned that perhaps there exists a morphine-like substance produced by the body. This research and the subsequent endorphin/enkephalin discovery is reviewed in this article.

Historical perspective

The fundamental concept in opiate activity is understanding the function of the opiate receptor, the site which recognizes and binds opioid drugs with multiple biochemical and physiological sequela. The concept of an opioid receptor arose from research on systems that were sensitive to the effects of narcotics. It was found that electrical stimulation of the guinea pig ileum or of the mouse vas deferens caused acetylcholine release in the ileum and norepinephrine release in the vas deferens. Morphine was found to inhibit the release of these transmitter substances, consequently inhibiting the electrically stimulated contraction. Further research showed that this action was stereospecific and was reversible by opiate antagonists.

Liebeskind produced analgesia by electrical stimulation of the mesencephalic central gray and the periventricular gray regions. This analgesia, shown to be reversed by naloxone, exhibited cross tolerance with morphine-induced analgesia.

Using highly specific opioid agonists and antagonists, binding sites in brain synaptic membranes and guinea pig ileal homogenates were demonstrated. These receptor sites were functional only with opioid drugs; drugs related to cholinergic, adrenergic, serotonergic and histaminergic systems were ineffective. Steroids and peptide hormones were also ineffective.

Several of these morphine-like substances were not at all like morphine. For example, their molecular weight was in the range of 800-1000. (Morphine has a molecular weight of 285.) Both leucine aminopeptidase and carboxypeptidase were found
to destroy activity. It became evident that these substances were in fact peptides.

In 1975, Hughes elucidated the structure of the morphine-like factor which had been previously named enkephalin. Enkephalin proved to be a mixture of two pentapeptides. One, (Met⁵)-enkephalin, had the sequence H₂N-Try-Gly-Gly-Phe-Met-OH and the other, (Leu⁵)-enkephalin, had the sequence H₂N-Try-Gly-Gly-Phe-Leu-H. It was demonstrated that synthetic (Met⁵)- and (Leu⁵)-enkephalins produced the full spectrum of opioid-like effects. Hughes also made the observation that the structure of (Met⁵)-enkephalin was contained within the sequence of a 91 amino acid known as beta-lipotropin (beta-LPH) isolated by Li in 1965. The function of this polypeptide was known only to be regulation of fat metabolism. Additional studies found this peptide to be present in all vertebrates and it is not present in any invertebrates.

To clarify nomenclature, the opioid peptides related to beta-LPH are known as endorphins. The term is analogous to the term corticotropin, which denotes a biologic activity rather than a specific chemical structure. The enkephalins are the specific pentapeptides identified by Hughes and belong to the endorphin class.

Further research with beta-LPH beginning with the number 61 amino acid, which starts the (Met⁵)-enkephalin pentapeptide, has identified other endorphins: alpha, gamma and delta. Li renamed the entire amino acid sequence beta-LPH 61-91, which was originally known as C-fragment, to beta-endorphin (for endogenous morphine).

### Biosynthesis and degradation

In order to understand the biosynthesis of beta-endorphin we must first look at the adrenocorticotropic hormone (ACTH). ACTH is produced by both the brain and the pituitary gland. The largest form of ACTH has a molecular weight of roughly 31,000 and is known as “big ACTH” or 31 KACTH. ACTH contains 39 amino acids and has a molecular weight of about 4,000.

Several pieces of evidence support the contention that big ACTH may function as a biosynthetic precursor to both ACTH and related peptides, and to beta-lipotropin and its related peptides. Such an example is found in the report of a particular pituitary tumor which produces peptides with opioid agonist activity. Additionally, staining the pituitary with antibodies to both ACTH or beta-LPH shows staining in the same cells of the pars intermedia and adenohypophysis. Thirdly, the release of beta-LPH and ACTH appears to be in parallel fashion with circulating concentrations of beta-LPH and ACTH rising and falling together in response to various physiological manipulations. Also, dexamethasone depressed both circulating ACTH and beta-LPH. Lastly, big ACTH has been shown to contain (as part of its amino acid sequence) the entire sequences of beta-LPH and ACTH.

Some research has been carried out to determine the metabolism of the various opioid peptides. The enkephalins are extremely and rapidly degraded by enzymes in various tissue homogenates or in blood; however, beta-endorphin is more stable. This would serve to explain the increased antinociceptive potency of beta-endorphin over (Met⁵)-enkephalin. In homogenates of the brain, enkephalin appears to be degraded primarily by removal of the N-terminal tyrosine.

### Distribution of opioid peptides

It is interesting to note that the globus pallidus has a concentration of enkephalins 5-10 times higher than any other area of the brain. Although the function of this concentration is not clear, it may be reasonable to believe that this is involved in the extra-pyramidal control of motor activity. Narcotics and synthetic or natural opioid peptides can produce either hypermotility or cataleptic effects, depending on dosage and other factors.

The limbic system also has a high concentration of enkephalin; the nucleus accumbens and amygdala contain large amounts. This distribution may provide a physiologic basis for mood alteration associated with morphine use. Such a role may implicate enkephalins in mental illness.

In the midbrain, concentrations of enkephalin are moderate to low with the exception of the periaqueductal gray matter (PAG) and raphe nuclei. These areas are known to be important in the transmission of pain signals. Reynolds showed electrical stimulation of the PAG produces analgesia in both animals and man, and in addition, microinjection of narcotics into this region produces analgesia. Mayer and Price demonstrated the importance of the raphe nuclei and associated serotoninergic systems in the mediation of pain sensations.

Outside the central nervous system (CNS), the greatest concentration of enkephalin is in the gastrointestinal tract. In humans, the endocrine cells that stained for enkephalin also stained for gastrin. The presence of enkephalin in the gastrointestinal tract fits well with the pharmacology of narcotics, that is, with the ability of morphine to produce constipation.
Neuroendocrine effects. The pituitary appears to be a storage/synthesis site for beta-endorphins. The effect of beta-endorphin levels on other pituitary hormones has been the focus of some research. It is known that morphine and other opioid drugs may increase the release of prolactin, growth hormone and vasopressin (ADH) following peripheral or intraventricular administration. The effects of the endogenous opioids vary with the analogue and route of administration, but in general, the opioid peptides cause the release of prolactin, growth hormone and vasopressin, although the release of luteinizing hormone (LH) and thyroid-stimulating hormone (TSH) are depressed.

Beta-endorphin is more potent in all aspects than (Met²)- or (Leu⁶)-enkephalin. This is consistent with the rapid metabolic breakdown of the pentapeptides. The effects of the opioid peptides are naloxone reversible, indicating that the effects are mediated by opioid binding sites. In the case of prolactin, the control of prolactin release appears to be due to inhibition of dopamine release in the hypothalamus, which then has a direct effect on the pituitary.

Nociceptive transmission

Stimuli which result in tissue damage produce pain. This damage can result from mechanical, thermal or chemical insult. Nerve terminals responding to noxious stimuli are terminals of slowly conducting myelinated axons of the A-delta group and the unmyelinated C fibers. Stimulation of the A-delta fibers by noxious stimuli produces a sharp prickling type of sensation whereas the C fibers transmit the more prolonged burning type of pain.

These fibers enter the CNS through the dorsal root of the spinal nerves and their terminals synapse with cells in the superficial layers of the spinal cord dorsal horn. The dorsal horn can be subdivided into layers based on the anatomical characteristics of each area. The most dorsal region is called lamina I. This region contains neurons which respond specifically to noxious stimuli and is the primary region of termination of the A-delta and C fibers.

These fibers also terminate in the underlying layers—laminae II and III known as the substantia gelatinosa. Lamina V contains large cells which respond with a low firing rate to touch and with a much higher discharge rate to pinch or pin prick. Therefore, the neurons of laminae I, II, III and V are involved in the transmission of noxious stimuli. They send axons up the spinal cord to higher centers in the brainstem and thalamus.

Opiate receptors have been found in the spinal cord and are concentrated in laminae I and II. Experimentation reveals that morphine could be acting postsynaptically on dorsal horn neurons or on presynaptic terminals of the A and C fiber primary afferents.

At most locations in the brain, microinjections of opioids do not produce analgesia, but marked analgesia is produced when the opioid is injected into the PAG and the lateral mesencephalic reticular formation and nucleus gigantocellularis. The PAG does not have a direct projection to the spinal cord; therefore, PAG-induced descending inhibition must be relayed via other neurons. A projection from the PAG to the nucleus raphe magnus, which is rich in opioid receptors, is known to project to the spinal cord dorsal horn. We have discussed the nociceptive pathway, but how do opioid peptides inhibit a noxious stimulus? Neurotransmitters and agonist drugs are conventionally thought to bind to receptor sites at synapses and trigger some change in membrane properties such as an alteration in ion permeability or cyclic nucleotide formation.

Studies of ionic influences on opiate receptor binding suggest mechanisms whereby opiate recognition at receptors is translated into altered ion permeability. Low concentrations of sodium selectively decrease the binding of opiate agonists and enhance the binding of antagonists. Sodium appears to accelerate the rate at which opiate agonists dissociate from the opiate receptor. Recent studies have shown that opiates and the enkephalins do indeed inhibit neuronal activity at some loci by decreasing sodium conductance, apparently by acting directly at the ion channel.

It would probably be misleading to think of opiate receptors as exclusively mediating analgesia, since the characteristic effect of the opiates in humans is less a specific blunting of pain sensation than the production of a peculiar state of indifference, an emotional detachment from the experience of suffering. This is consistent with the high distribution of opioid receptors in areas of the brain affecting mood.

Endogenous analgesia

Soon after Hughes elucidated the structure of enkephalin, synthetic pentapeptides were injected into mouse and rat brain and some transient analgesic effects were observed. We now know that these transient effects were due to the rapid metabolic breakdown of the pentapeptides. When stable compounds such as beta-endorphin or modified analogues of the enkephalins were used,
the results were more sustained. Comparing analgesic activity of beta-endorphin to that of \( \text{Met}^5 \)-enkephalin, the former is several thousand times more active in spite of the fact that the two compounds have similar affinities for the opiate receptor.

In a study involving 30 patients with chronic intractable pain, intracerebral stimulators were implanted for pain relief. Under local anesthesia, multiple cerebral spinal fluid (CSF) samples were obtained every five minutes for 30 minutes. The level of enkephalin-like material rose from a baseline of 0.68 ± 0.2 picomoles/ml to a level of 0.88 ± 0.3 picomoles/ml at the end of 25 minutes of stimulation. The baseline levels exhibited by these patients were significantly lower than normative levels (3.1 picomoles/ml).

In a recent study, Oyama selected 14 patients with chronic intractable pain in the back, chest, abdomen, rectum and thigh secondary to metastatic malignancies. After intrathecal administration of 3 mg of synthetic beta-endorphin at the L2-L3 interspace, profound and long lasting analgesia (mean duration of pain relief 33.4 hours) was produced. No respiratory depression, hypotension, hypothermia or catatonia was observed.

In a study involving "on demand" pain relief, CSF levels of endorphins were measured in patients who were fitted with a programmable injection device for delivery of pethidine (meperidine). The study showed those patients with low endorphin levels required more analgesia; those patients with endorphin levels of 20 picomoles/ml required approximately 150 mg of pethidine, whereas patients with endorphin levels of 10 picomoles/ml required from 250-350 mg of pethidine.

Beta-endorphin levels have been found to increase during the progress of labor in a group of women having induced labors, without systemic analgesia during labor. Levels rose six times from the control period to the post-partal period. On examination of human placenta, two beta-endorphin-like peptides have been noted; however, these differ from beta-endorphin by being 12 amino acids longer than the pituitary hormone. Houck hypothesizes that the physiologic function may mediate the pain of parturition.

The identification of endogenous opioids provides a physiologic basis for acupuncture. The analgesic effect of acupuncture appears to be the result of interaction between impulses from the site of pain origin and afferent impulses from the acupuncture point, with this interaction taking place at different levels of the CNS. For acupuncture to be effective in relieving pain, the nucleus raphe magnus and endogenous opiates must be involved, as the analgesic effect of acupuncture is abolished by the administration of naloxone.

All potent analgesics exhibit the phenomena of tolerance and dependence. The endogenous opiate system now provides an explanation of this. Under resting conditions, opiate receptors are exposed to a certain basal level of enkephalin. Administered morphine binds to usually unoccupied receptors, thereby potentiating the analgesic effects of the enkephalin system. On sustained treatment with morphine, cells that have opiate receptors find themselves overloaded with opiate-like material, and by a hypothetical feedback loop, they send a message to enkephalin neurons to stop releasing enkephalin. When this happens, the binding sites are exposed only to morphine and can tolerate more morphine to make up for the enkephalin they are no longer receiving. When the administration of morphine is stopped, the opiate receptors find themselves with neither morphine nor enkephalin, and this lack initiates a sequence of events that results in withdrawal symptoms.

Other effects

Depression: The opiate peptides have been shown to decrease the turnover of serotonin and dopamine, substances which are thought to be concerned with mood. An abnormal amount of CSF endorphin has been measured in patients with schizophrenic and manic-depressive psychosis.

Vomiting: Opiate receptors are concentrated in the area postrema of the brain stem which includes the chemoreceptor trigger zone. Morphine seems to exert some of its central actions by interfering with dopaminergic transmission and initiating a mechanism which resembles denervation supersensitivity.

Sexual function: When inoculated into the lateral ventricles of rats exposed to estrous females, beta-endorphin enhanced the number of mountings; this excitatory effect can be inhibited by naloxone.

Constipation: Exogenous and endogenous opiates can reduce or abolish peristaltic activity of the isolated intestine induced by electrical stimulation or through increased intraluminal pressure.

Oliguria: Oliguria frequently occurs before a migraine attack and terminates with compensatory polyuria after the attack is over. An opiate substance of unknown structure and low molecular weight has been extracted from the plasma of migraine victims. This substance produces pro-
longed analgesia, reversible with naloxone, when injected into rat PAG. Because opiates stimulate the release of antidiuretic hormone (ADH) from the posterior pituitary gland, the morphine-like factor could participate in the modulation of hormonal antidiuresis and the fluid retention in migraine attacks could be due to a release of ADH.²⁷

Conclusion

The study of the endogenous opiates will have an impact on every discipline in medicine. The research has been voluminous as evidenced by the more than 3,500 articles published in the last four years. Conjectures of how the specialty of anesthesia may benefit are obvious—the result could be a new class of analgesics without the annoying side effects of the morphine class of opiates or possibly, a new class of hypnotics. Whatever the outcome, practitioners and patients alike will profit from the increased knowledge and understanding of a complex physiologic system which was virtually unknown six years ago.

REFERENCES


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