REVIEW ARTICLE

ENDOGENOUS OPIATE : CHARACTERIZATION AND PHYSIOLOGICAL ROLE IN PAIN MODULATION

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The hypothesis that opiates exert their pharmacological actions by interacting with the specific receptor sites located on the surface or inside the nerve cells in the brain and possibly other tissues has been proposed since the last decade. The reason for postulating receptors for morphine and the related natural and synthetic narcotics is the finding that all these opiate agonists, or analgesically active compounds, show basic similarities in their molecular structures. A slight modification of this basic structure could result in an increase or a decrease in potency, or could transform the compound to the antagonist. In addition, a high degree of steric and structural specificity inherent in many of the actions of the opiates observed,i.e. for a large number of morphine-like analgesics studied, it is always the levorotatory (-) isomer that is active, whereas the dextrorotatory (+) isomer has little or no analgesic or any other actions associated with opiates. Moreover, all pharmacological effects of an opiate could be obtained with a very small dose of the drug, in turn suggesting an interaction of the drug with the highly selective receptor sites.

The discovery of opiate receptors in the brain had led to a search for an endogenous morphine-like substance that would act as a natural opiate receptor ligand. There is evidence for at least five opioid peptides in mammalian nervous tissues: methionine- and leucine-enkephalins, β-endorphin, dynorphin and most recently α-neo-endorphin.

This article describes the experiments carried out by a number of laboratories to characterize the endogenous opiates, and to implicate their physiological role in the modulation of pain perception.

Enkephalins

Hughes (1) observed an opiate-like activity of the extract of a low molecular weight substance from the brain of rabbits, guinea-pig, rats and pigs. The opiate-like substance had been isolated and identified (2,3) and found to consist of two similar pentapeptides with sequences of H-Tyr-Gly-Gly-Phe-Met-OH (Methionine-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (Leucine-enkephalin). The existence of these two opiate peptides has been confirmed by Pasternak et al (4) and Simantov and Snyder (5). It has been found that bovine brain contains 4 times as much leu-enkephalin as met-enkephalin whereas in pig brain this ratio is reversed. The competition for opiate receptor binding by leu-enkephalin suggests that leu-enkephalin may be a "pure" agonist than met-enkephalin. The structure of met-enkephalin is contained within the sequence (residues 61 to 65) of 91 amino acids of β-lipotropin, a peptide isolated from the pituitary gland of several vertebrate species (6,7).

In the studies of Hughes and coworkers (1,2), it was found that both met- and leu-enkephalins had potent agonistic action at opiate receptor sites in that they produced a dose-related inhibition of electrically evoked contraction of the mouse vas deferens and the guinea-pig ileum preparations. These inhibitory effects could be completely antagonized by naloxone. Met-enkephalin is about twenty times more active than normorphine in the mouse vas deferens and equipotent to normorphine in the guinea-pig ileum. With the modified purification procedure, enkephalins were found unevenly distributed in the brain (pig, rat, rabbit) the highest concentrations occurring in the striatum, midbrain, pons and medulla. No enkephalins were detected in cerebellum, liver and lung (1).

The regional distribution of enkephalins in the monkey brain has been examined by the inhibition of ^{3}H-naloxone binding to rat brain membranes (8). It has been found that the regional distribution of enkephalin activity in the monkey brain resembles in general with the regional distribution of opiate receptor binding. However, the most marked discrepancies between the distribution of enkephalins and the opiate receptors involve the amygdala which contains the highest density of opiate receptor binding in the monkey brain but only moderate to low levels of enkephalins. The periaqueductal gray, one of the areas most enriched in opiate receptor binding, also contains only moderate levels of enkephalins. The medial thalamus, implicated in the mediation of affective components of pain, is highly enriched in opiate receptor binding and contains about three times as much opiate receptor binding as the lateral thalamus. Enkephalin activity in the medial thalamus is fairly low, but is more than twice of the levels in the lateral thalamus (8). However, the correlation of the regional distribution of enkephalin activity and that of opiate receptor binding in the brain has been reported in rabbits (1), calves (4,9) and rats (4,9), which in turn suggests the possible roles of enkephalins in the modulation of pain perception.

β-Endorphin

Simultaneously with the discovery of enkephalins, a 31-amino acid peptide with opiate-like properties, β-endorphin has been isolated and identified from the pituitaries of bovine (10), porcine (10,11), camel (12), and human (13,14). The amino acid sequence of β-endorphin is identical to the sequence of the carboxy terminal 31 amino acids of human β-lipotropin (i.e. β-LPH61-91). Immunohistochemical studies show a dense staining for β-endorphin-containing cells in pars intermedia, with a sparser distribution of immunoreactive cells in the adenohypophysis, the neurohypophysis appears devoid of such activity (15).

In the rat brain, the β-endorphin-like activity has been found to be about 3-4 times lower than in the pituitary gland (16). High β-endorphin-like immunoreactivity is found in the medial hypothalamus, where it is threefold that in the next higher brain areas (preoptic region and mesencephalon) and nearly fivefold the value in the lateral hypothalamus. Moderate β-endorphin-like immunoreactivities are present in the amygdala, septum and nucleus accumbens. Despite the high enkephalin level in the striatum, only low β-endorphin-like immunoreactivity is found in this area, especially in the globus pallidus. The thalamus, the cerebral cortex as well as the hippocampus also contain low β-endorphin levels (16).

It has been reported that β-endorphin, adrenocorticotropin (ACTH), β-MSH and α-MSH are all formed from a common precursor of 28-41K Daltons which has been termed pro-opiocortin (17-20). Furthermore, ACTH and β-endorphin are released concomitantly after stressful stimuli in rats such as footshock (21) or leg break (22). The association between β-endorphin and ACTH might well reflect the proposed common origin and biogenesis of these two peptides. Footshock stress in rats can also elicit an analgesic response which is partially reversed by naloxone (23-25). It is possible that β-endorphin has an unknown peripheral physiological role, perhaps related to changes in adrenocortical function or intermediary metabolism during stress. The presence of opiate receptors in the pituitary (26) also reflects the possible intrapituitary function of β-endorphin which could involve in the modulation of hormone synthesis or release. Alternatively, β-endorphin may be released into the hypothalamic portal system (27) and be retrogradely transported so that it could act on hypothalamic or other brain centers(28).

Dynorphin and α-neo-endorphin

Goldstein et al (29) have partially sequenced a peptide from commercial pituitary powder that contains the leu-enkephalin sequence

with a basic carboxy terminal extension. This peptide, named dynorphin has its first 13 residues as Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys. It appears to be remarkably potent in the guinea-pig ileum assay, being some 730 times that of morphine and 54 times that of β-endorphin. Its effects on this tissue are blocked completely by naloxone, but the apparent affinity of naloxone is 1/13th that for blockade of leu-enkephalin or normorphine. In the mouse vas deferens, dynorphin (1-13) is three times more potent than leu-enkephalin. In the pituitary (porcine, bovine and rat), immunoreactive dynorphin is found predominantly in pars nervosa. In the central nervous system, it is distributed widely, with high concentrations in the hypothalamus, the medulla-pons, the midbrain and the spinal cord (30). In rabbits and rats, the concentration of immunoreactive dynorphin is high in the dorsal aspect of the spinal cord ,but is low in the root ganglia of both species (31)

Millan et al (32) observed the distribution of immunoreactive -dynophin (ir-dyn) in the pituitary, discrete regions of brain and the spinal cord, and observed the influence of a 5 min footshock stress upon levels of ir-dyn in these structures in rats. It was found that footshock produced a significant fall in the anterior pituitary lobe content of ir-dyn but no significant change in its neurointermediate lobe. In the hypothalamus, a significant elevation in level of ir-dyn was observed. With the exception of the frontal cortex, in which a decrease in level of ir-dyn was found, in all other brain regions examined no significant changes emerged. A significant diminution in concentrations of ir-dyn in both the lumbosacral and thoracic sections of the spinal cord was also detected. These authors suggested that, due to a particularly high potency of dynorphin (29), the changes observed in their experiments might be of considerable functional significance in the response to stress (32).

Wuster et al (33) employed the technique of chronic opioid infusion to produce tolerance attributable to specific receptor population in the mouse vas deferens. They observed that the mouse vas

deferens preparations taken from mice previously simultaneously infused with D-Ala^{2}D-Leu^{5}enkephalin (a δ-agonist) and sufentanyl (a μ-agonist) for a period of six days, developed tolerance to δ-agonist and μ-agonist action. However, it was observed that such tolerance did not develop to the activity of dynorphin (1-13). These authors therefore suggested the existence of highly selective dynorphin receptors in the mouse vas deferens, which might function independently of the μ- and δ-receptors in this tissue. Friedman et al (34) demonstrated the significant effect of dynorphin(1-13) on morphine and β-endorphin-induced analgesia after the intracerebroventricular injection in mice (the tail-flick test). They observed that dynorphin(1-13) itself did not produce any significant analgesia. This peptide attenuated the analgesia produced by all doses of morphine used. The peptide attenuated the analgesic effect of high doses of β-endorphin whereas it potentiated that of the low doses, suggesting some common sites might exist between β-endorphin and dynorphin(1-13).

Pierce et al (35) observed an analgesic activity of dynorphin (1-13) by the tail-flick test but not by the hot plate test and only after the intraspinal administration, not either after the intracranial or the intraperitoneal administration. In the same experiment, ethylketocyclazocine was found to be most potent by the intraspinal route irrespective of the analgesic tests. The finding that dynorphin relied solely on the spinal sites for its analgesic activity and ethylketocyclazocine, a κ-receptor agonist also showed a preference on the spinal cord activity had led the authors (35) to suggest that dynorphin(1-13) might be an endogenous ligand for the κ-receptor. This hypothesis has been further supported by Chavkin et al (36) who demonstrated that dynorphin(1-13) and ethylketocyclazocine had equally poor sensitivity to naloxone antagonism on the guinea-pig ileum preparation. Moreover, in the binding assays with the membranes from guinea-pig brains, both compounds were more potent in displacing ^{3}H-ethylketocyclazocine than in displacing μ- or δ-opiate receptor ligands

Kangawa and co-workers (37,38) have recently isolated and identified a new opiate-like decapeptide, α-neo-endorphin from porcine hypothalamus. The sequence of this peptide has been determined to be Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys. This peptide showed a very potent opiate activity in the guinea-pig ileum assay, 6.7 times as high as met-enkephalin and 5 times as high as β-endorphin.

It seems, from the studies of β-endorphin, dynorphin and α-neo-endorphin, that, chemically speaking, there are at least two families of opiate peptide based on met-enkephalin and leu-enkephalin respectively. It also appears that carboxy-terminal extension can greatly enhance potency of both enkaphalins. In all three cases, β-endorphin, dynorphin and α-neo-endorphin have a number of strongly basic side chains. Carboxy-terminal extension employing neutral amino acids does not give this enhanced potency (40).

Specific receptor sites for endogenous opiates

The relation of the endogenous opiates, enkephalins and β-endorphin, to the opiate receptors has been established by the multiple parallel assays in the laboratory of Lord et al (41). Two of the assays were pharmacological in which the depression of the electrically induced contractions in the guinea-pig ileum and in the mouse vas deferens were observed. The other two were based on the inhibition of the binding of ^{3}H-leu-enkephalin and opiate antagonist ^{3}H-naloxone, in homogenates of guinea-pig brain. It was found that β-endorphin is equipotent in the two pharmacological models and also in the two binding assays. Leu-enkephalin behaves differently in that it is much more potent in the mouse vas deferens than in the guinea-pig ileum and also more effective in inhibiting ^{3}H-leu-enkephalin binding than in inhibiting ^{3}H-nalaxone binding. The activity pattern of met-enkephalin is intermediate between those of β-endorphin and leu-enkephalin. In contrast, morphine is more potent in the guinea-pig ileum than in the mouse vas deferens and

is also a better inhibitor of ^{3}H-naloxone binding than of ^{3}H-leu-enkephalin binding. It was concluded that the receptors preponderant in the mouse vas deferens (δ-receptors) corresponded to the leu-enkephalin binding sites in the brain and that those preponderant in the guinea-pig ileum (μ-receptors) were correlated to the naloxone binding sites (41).

In conclusion, a number of studies (e.g.41-43) have clearly defined at least three receptor types of which none of the ligands (natural and synthetic) is absolutely specific for one receptor type but preferences are clearly discernable in various bindings or bioassays. At the present time, the μ-receptor is defined as having a preference towards morphine, dihydromorphine and β-endorphin as agonists and naloxone as an antagonist. The δ-receptor has a greater affinity for the enkephalins as well as β-endorphin but a poor affinity for naloxone. The κ-receptor is less well defined but apparently prefers compounds of the ketocyclazocine series and only has a weak affinity for naloxone. Specific receptor blocking agents are not available for probing these different receptor types, i.e. naloxone does has a high affinity for the μ-receptor but it is not specific in that it will also interact with the δ-and κ-receptors.

Possible physiological role of endogenous opiates in the modulation of pain perception

The physiological role of endogenous opiates in the modulation of pain perception has been proposed by a number of investigators who employed various experimental techniques, such as naloxone antagonism, microinjection of the compounds into various brain areas, electrical stimulation of the brain, acupuncture, stress-induced analgesia, etc.

Jacob et al (44) provided the first evidence in experimental animals (mice and rats) that naloxone produced hyperalgesia and hypothesized that this was due to the interference of the natural occurring substances that are physiological regulators of pain sensitivity and

reaction to pain. These observations have been confirmed by some laboratories, (e.g.45-47), but not by others (e.g.48-50) possibly because of slight differences in methodology. Experiments with naloxone injection into humans have also been investigated. El-Sobky et al (51) found no reduction in pain thresholds in a situation where pain was induced acutely by electrical stimulation of the skin in the healthy volunteers. Naloxone was administered on a double blind basis, and the reaction to ischemic arm pain measured. No effect on pain thresholds was observed whereas the subjects reported slight changes in mood and feeling, and an increase in anxiety, hostility and depression, which could in fact have due to a blockade of endogenous opiate activity. Morphine is known to affect mainly the reaction to pain tolerance rather than pain thresholds, and the blockade of endogenous opiates would be expected to affect this psychosomatic component.

It has been well documented that the mesencephalic periaqueductal periventricular gray (PAG) regions in the midbrain appear to be the major sites for opiate analgesic activity in the CNS. Electrical stimulation of these regions or the nearby regions could result in an analgesic response in animals and human (52-56). Such stimulation produced analgesia are partially or completely reversed by naloxone (53-56). Furthermore, it has been demonstrated that the stimulation produced analgesia outlasts the brain stimulation for a few minutes to several hours, depending on the duration and intensity of the stimulation (52,57,58). This observation may suggest that the electrical stimulation of the medial brainstem appears to produce analgesia by activation of an endogenous pain inhibitory system, and the endogenous opiate system appears to be the most potential candidate for this phenomenon. This hypothesis has been supported by the observation of the opiate agonistic effects, particularly analgesia and catatonia, in animals which have been microinjected with enkephalins (leu-and met-enkephalins) or β-endorphin into the periaqueductal gray regions (e.g.59-61).

Mayer et al (62) observed that the pain threshold to electrical stimulation of the tooth was significantly increased by acupuncture needling of Hoku points between the thumb and index finger. This increase was completely reversed by naloxone 5 min after injection. The reversal was incomplete 10 min after the injection and disappeared within 15 min. In contrast, these authors found no effect of saline injection on analgesia produced by acupuncture and no effect of naloxone on the base-line pain threshold. The reversal effect of naloxone on acupuncture analgesia in human has been reported by many investigators (e.g.63,64). These results suggest the possible involvement of endogenous opiates in acupuncture analgesia.

It has been accepted that animals which are submitted to a stressful procedure exhibit antinociceptive (analgesic) response. The antinociception induced by stressful events was linked to the brain endogenous opiates because acute exposure to stress induced changes in levels of brain opiate peptides (23,24) and changes in brain opiate receptor binding characteristics (65,66). It was also noted that β-endorphin and ACTH were found to be concomitantly released from the pituitary gland following an acute stress in rats (21,22). Rossier et al (67) found a decrease in leu-enkephalin levels in hypothalamus of rats following footshock. No changes were observed in other parts of the brain. These data provided evidence in support of the involvement of endogenous opioids in stress-induced antinociception. On the other hand, there is evidence which casts doubt on this hypothesis. For example, the evidence provided by Rossier et al (21) who reported an increase in β-endorphin level in the blood but not in the brain following footshock in rats. Furthermore, Fratta et al (68) found no change in the met-enkephalin content of the whole brain of rats after footshock. A lesion placed in the dorsal part of the lateral funiculus of rat spinal cord, which reduced or abolished morphine and electrical brain stimulation-induced analgesia (69) , had no effect upon analgesia induced by electrical footshock (70)

Similar observations have arisen from a number of studies which used morphine and its antagonist naloxone, as the experimental tools to implicate endogenous opiates in stress-induced antinociception. Stress-induced antinociception was found to be fully or partially reversed by naloxone in some studies (e.g.23,25,47,50,71-75),but not in other (25,75-77). The cross tolerance between morphine and stress-induced antinociception has been reported by some observers (50, 74,75,78,79), but again not by others (80,81).

Tolerance to and physical dependence on endogenous opiates

Simultaneously with the observation in the experimental animals of the analgesic activity of an intracerebroventricular dose of enkephalins and β-endorphin (e.g.59-61) an attempt has been made to investigate whether repeated administration of the endogenous opiates would lead to tolerance and physical dependence in the same manner as observed in exogenous opiates (82-87).

The antinociceptive effect of human β-endorphin after an intracerebroventricular injection observed in rats was found to be 21 times more potent than morphine (85). This peptide also produced morphine-like catatonia and hypothermia. The responses were blocked by naloxone. Repeated injection of the peptide (94 μg twice daily for 2 days) induced tolerance to analgesic response, catatonia and hypothermia. Furthermore,a cross-tolerance to morphine was also observed. The similar results were also reported by Van Ree et al (82).

Loh et al (83) investigated an analgesic activity of β-endorphin after an intracerebroventricular injection, which was found to be 18 to 33 times more potent than morphine and its action was blocked by naloxone. The authors found that met-enkephalin produced a weak analgesic effect of short duration (less than 5-10 min) after an intracerebroventricular injection. After 70 h of infusion of β-endorphin into the rat brains, all the animals demonstrated a naloxone-precipitated

morphine-like withdrawal syndrome. Such results were also observed with met-enkephalin (84)

In the experiment of Huidobro-Toro and Leong Way (86) a β-endorphin injected intracerebroventricularly in mice produced a rapid onset, dose-dependent antinociceptive effect. Single dose tolerance development was demonstrable with doses twice or more than the LD50 (median analgesic dose). Tolerance was maximal at about 12 h following the priming dose and disappeared within 48 h. Tolerance was accompanied by some degree of physical dependence as noted by signs of naloxone-precipitated withdrawal similar to those elicitable in the morphine-dependent state. Tolerance development to β-endorphin was blocked by the simultaneous administration of naloxone and also by pretreatment with actinomycin D or cycloheximide (the protein synthesis inhibitors). The authors (86) concluded that the single dose tolerance to β-endorphin appeared to be initiated by a process similar to those involved with tolerance resulting from chronic administration of morphine.

Tseng (87) observed the analgesic action of D-Ala^{2}D-Leu^{5}enkephalin (DADL) in rats with the tail-flick test, and reported that a chronic intrathecal infusion of DADL for 5 days caused a shift of dose response curves of both DADL and morphine injected intrathecally to the right. This result indicated that a tolerance to DADL analgesic action and cross-tolerance to morphine had developed in the chronically DADL infused animals. However, concomitant intrathecal infusion of naloxone which was more sensitive in blocking μ-receptors than δ-receptors blocked the development of cross-tolerance to morphine while that to DADL was left unaffected. This author (87) presented the evidence that two types of opiate receptors, δ- and μ-receptors, in the spinal cord of rats were involved in the development of tolerance by chronic DADL exposure.

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