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# ENDOGENOUS PEPTIDES AND ANALGESIA

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## INTRODUCTION

Endogenous analgesic mechanisms were recognized and utilized in early times when pain was treated with acupuncture or other types of "counter-irritation" (1) using chemical irritants, mechanical devices, or extreme temperatures. Electrical stimulation, used as early as the nineteenth century to alleviate pain during tooth extractions (2), was later considered obsolete and never studied scientifically. When Reynolds (3) showed that electrical stimulation of the mesencephalic central gray matter produced surgical analgesia, this represented a breakthrough for a modern scientific approach to pain relief via endogenous mechanisms. His observations were soon confirmed by others (4), and speculations regarding the mechanism of action followed.

The observations of Murray & Miller (5), suggesting analgesic principles in pituitary extracts, did not attract much attention. The existence of a humoral endogenous analgesic was also intimated by Collier (6). However, the starting point for the search for endogenous analgesics in 1974–1975 was the publication, one year earlier, of reports that opiates combine with specific receptors in brain and nerve plexi of intestine (7–9). These receptors showed specificity in that only narcotic analgesics and their antagonists were bound to an extent proportional to their biologic potencies while other neuroactive agents, drugs and neurotransmitters alike, showed no receptor affinity. Teleologically it seemed unlikely that opiate receptors should have evolved for the sole purpose of combining with plant alkaloids, and the search for endogenous opiate receptor ligands began. Reports from two laboratories (10–11) gave evidence for the presence of *peptide* opioids. The publication in December 1975 of the structure of the enkephalins (12), pentapeptides with opiate receptor affinity, gave a sound chemical background for further work in the field, but soon several endogenous morphine-like peptides were found to exist (13–16). These peptides, i.e. enkephalins and endorphins, are now, following the suggestion of Eric Simon, being collectively called endorphins.

The endorphin field is rapidly expanding and has already been the subject of many reviews (17–21). The present review is concerned specifically with endorphins and pain mechanisms.



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## *General Characteristics of the Endorphin Systems*

The enkephalins, first extracted from brain, were also found in nerve plexi of the gastrointestinal tract (10) and more recently in exocrine cells of the stomach and intestine (22). The α- and γ-endorphins have been isolated from hypothalamic-pituitary tissue (15), while β-endorphin has been characterized in extracts of the pituitary gland (16, 23). Interestingly, methionine-enkephalin and the α-, β-, and γ-endorphins may all be regarded as fragments of the C-terminal part of the pituitary hormone β-lipotropin (Figure 1). It has therefore been inferred that β-lipotropin serves as a prohormone for the endorphins (16, 24). This may be so for the pituitary endorphins (although no proof is available) but is not so likely for the enkephalins in the brain (see below). In fact, several observations point to the existence of two major, separate endorphin-releasing systems, one localized mainly in the pituitary with β-endorphin as the principal active agent, the other localized in the brain where enkephalins are abundant.

The endorphins of the pituitary are found and likely produced in the pars intermedia and pars distalis (25–26). The most important pituitary endorphin is probably β-endorphin which is comparatively stable in blood and is active as an analgesic when injected intravenously (27). The pituitary endorphin system may therefore exert sustained hormonal control. Comparatively small quantities of β-endorphin are present in brain (about 15% of total pituitary content). In neural tissues the major identified endorphins are the enkephalins, distributed over wide areas of the CNS and in peripheral nerve plexi. There is a reasonably good correlation between the distribution of opiate receptors measured by various techniques and the presence of enkephalin fibers (28–29). Antibodies to both the enkephalins and to the longer endorphin peptides have been used to localize the endorphins in histological sections using the indirect fluorescence technique of Coons (30). Although this approach has its pitfalls (unspecific staining, cross-reacting antibodies, etc) several carefully controlled studies have given consonant results. Enkephalin terminals are observed in many areas of the rat CNS (31) including those thought to be of importance to pain and analgesia (the dorsal horn of the spinal cord, the spinal trigeminal nucleus, the raphe nuclei, and the periaqueductal gray matter). Terminals are also seen over the limbic system and in basal ganglia. With optimal experimental conditions, enkephalin reactive cell bodies can also be demonstrated (32), allowing a preliminary mapping of enkephalin pathways in the CNS (see below). The enkephalin-reacting antibodies did not stain the pituitary, whereas antibodies directed against the longer endorphins react strongly with pituitary structures (25, 33), and in brain, only hypothalamic areas are faintly stained, apparently resulting from reaction with fibers other than those reacting with enkephalin-directed antibodies (33). Thus, several observations support the concept of a separate enkephalin-reacting system which probably does not have α-, β-, or γ-endorphin as precursors.

Endorphins can also be measured in human CSF (34) and in blood (35). The chemical nature of these endorphins is presently not fully established but the principal ones are not identical with previously described endorphins (36). Analysis of endorphins in CSF has been used extensively to study endorphin systems in man (37)

| Peptide | |
|---|---|
| Leu-enkephalin | H-TyrGlyGlyPheLeu-OH |
| Met-enkephalin | H-TyrGlyGlyPheMet-OH |
| α-Endorphin | H-TyrGlyGlyPheMet-ThrSerGluLysSerGlnThrProLeuValThr-OH |
| γ-Endorphin | H-TyrGlyGlyPheMet-ThrSerGluLysSerGlnThrProLeuValThrLeu-OH |
| | H-TyrGlyGlyPheMet-ThrSerGluLysSerGlnThrProLeuValThrLeuPheLysAsnAlalleValLysAsnAlaHis-OH |
| β-Endorphin | H-TyrGlyGlyPheMet-ThrSerGluLysSerGlnThrProLeuValThrLeuPheLysAsnAlalleValLysAsnAlaHisLysLysGlyGln-OH |

*Figure 1* Structure of porcine endorphins. All peptides except Leu-enkephalin have a common sequence (61–65 of β-lipotropin). β-Endorphin corresponds to the C-terminal sequence (61–91) of β-lipotropin.

Very little is known about the biosynthesis of endorphins in brain or pituitary. It also remains to be established which endorphin(s) is/are functionally important in brain or in pituitary secretions. The identification of these endorphins and studies regarding the regulation of their synthesis, turnover, and breakdown are strongly needed.

## PHYSIOLOGY OF ENDORPHINS IN RELATION TO PAIN

### *Neuroanatomical Distribution*

The anatomical localization of enkephalin pathways in areas related to pain and analgesia can be summarized as follows (38). Enkephalin-positive cell bodies and terminals are observed in the dorsal horn of the spinal cord, in the marginal layer and substantia gelatinosa of the spinal trigeminal nucleus, in the raphe magnus, and in the periaqueductal central gray. Rhizotomy or sectioning of the cord does not affect the nerve terminal density. The enkephalin neurons of the spinal cord and possibly the spinal trigeminal nucleus may therefore be interneuronal, and there is no evidence of ascending or descending enkephalin pathways in the spinal cord. There is no evidence for enkephalin-positive fibers in the primary afferents. At all levels there is a close association between enkephalin neurons and substance P terminals (38).

Morphine produces behavioral analgesia when microinjected into a few distinct areas, such as the periaqueductal gray matter and the raphe magnus nucleus, suggesting that morphine and allied drugs have a localized site of action (e.g. 39–41). Morphine also causes behavioral analgesia when injected into the spinal cord (42). Thus there is a good anatomical correlation between the location of enkephalin terminals and sites responding to morphine with analgesia (receptors) (Figure 2).

There is no evidence for the presence of enkephalins in the pituitary where the longer endorphins are found mainly in the pars intermedia and pars distalis (25–26).

### *Possible Functional Role of Endorphins*

The discovery of the endorphins is one of those unexpected events in physiology. Very few experimental observations seemed to indicate or have been interpreted to indicate their presence. For instance, the apparent inactivity of naloxone, except as a narcotic antagonist (43), seemed to preclude their existence. With the new biochemical developments a reassessment became necessary. First, if endorphins are morphine-like in every respect, and so far there are no observations to the contrary, we may deduce from the pharmacological profile of morphine which effects to investigate. Morphine acts on the CNS to cause analgesia, drowsiness, mental clouding, and changes in mood (44). Most studies have, naturally, been directed to pain and analgesia. If endorphins are normally present, morphine antagonists should produce effects opposite to those produced by morphine. It must be emphasized that much of the data obtained using the latter approach to support the concept of the role of the endogenous ligand is circumstantial.

Jacob et al (45) provided the first evidence in experimental animals showing that naloxone produces hyperalgesia and hypothesized that this was due to interference with naturally occurring substances that are physiological regulators of pain sen-

sitivity and reaction to pain. Mice and rats were found to become slightly hyperalgesic after naloxone. The effect was observed as an increased tendency to escape painful heat (the hot-plate technique). Although these studies have been confirmed by some (46), they have not been confirmed by others (47), possibly because of slight differences in methodology (cf 48). At any rate, these observations in animals indicate that the tonic activity of the endorphin systems is quite low. Experiments with naloxone injections into humans have also been largely negative. El-Sobky et



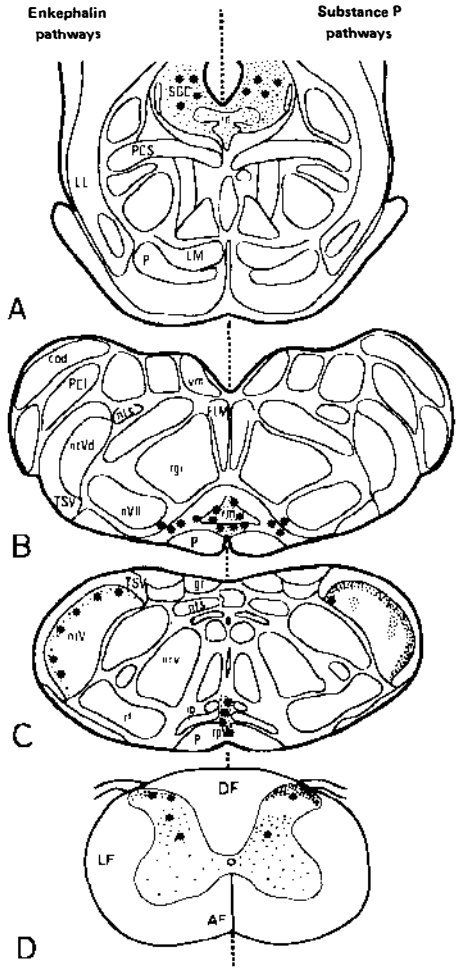


*Figure 2* Graphic presentation of areas related to pain and analgesia with enkephalin or substance P pathways at three levels of the lower brain stem and in the spinal cord in rats. *Dots* represent terminals, *asterisks* cell bodies. [Reprinted from (38) with permission.]

al (49) found no reduction in pain thresholds in a situation where pain was induced acutely by electric stimulation in paid healthy volunteers. The subjects reported practically no significant changes after naloxone was given on a double-blind basis. Grevert & Goldstein (48) recently reported similar results obtained using healthy volunteers. Naloxone was administered on a double-blind basis, and the reaction to ischemic pain measured. No effects on pain thresholds were observed. However, the subjects reported slight changes in mood and feelings and an increase in anxiety, hostility, and depression, which could, in fact, have been due to a blockade of endorphin. Morphine is known mainly to affect the reaction to pain (pain tolerance) rather than pain thresholds, and blockade of endogenous opioids would be expected to affect this psychosomatic component.

In early clinical studies with naloxone, reported objective and subjective psychic responses were mild (49). Several later studies with both naloxone and the related narcotic antagonist, naltrexone, largely confirmed these observations (e.g. 50) and further support the notion that in the normal individual, tonic release or efficacy of endorphin is low. On the other hand, in a pilot study of hallucinating schizophrenics, naloxone administration was followed by a reduction or obliteration of hallucinations (51).

Naloxone has also been tested on other physiological roles of endorphins. In acute spinal cats, naloxone increases spinal reflex activity, an action opposite to that obtained with morphine (52). Tachyphylaxis developed rapidly, suggesting that compensatory adaption had occurred. A rat can be conditioned to respond with hyperthermia to an olfactory stimulus if morphine is coadministered during the conditioning period. When morphine is withdrawn, hyperthermia can still be induced by the olfactory stimulus and this effect is naloxone-reversible (53), an observation that might be explained by conditioned stimulation of endorphin release. In this context, the paradoxical intensitivity to pain experienced during shock or severe trauma (54) could, at least partly, be a consequence of endorphin activation. Unfortunately such situations are difficult to study experimentally and endorphin release can only be suggested as one of several explanations. One study (55) actually implies increased endorphin activity in rats subjected to repeated sessions of inescapable foot shocks. Following a 30-minute session the animals were less prone to react to radiant heat, and when their brains were analyzed for total endorphin content there was an indication it had increased. Repeating the sessions on each of 13 days resulted in tolerance to both the biochemical and analgesic responses seen on day 1. None of the experiments described above are completely satisfactory for elucidation of the physiologic role of endorphins in pain and analgesia. The relation between experimental pain and clinical pain is uncertain. As has been emphasized above, morphine acts rather on the reaction to pain (pain tolerance) than on sensory thresholds. Experiments that require severe stress or trauma conflict with what is ethically permissible. Evaluation of patients suffering from trauma after operations or accidents also offers problems such as placebo reactions. A biochemical approach that provides more direct evidence is the measurement of endorphin levels in lumbar cerebrospinal fluid (CSF) from patients with various diseases (34, 37). The chemical nature of the active material is not yet fully established, but it is distinctly different

from that of the previously characterized endorphins (36). As shown in Table 1 there is a narrow range of endorphin values in healthy volunteers and in patients without pain undergoing routine neurologic examination. On the other hand, in patients with severe, chronic pain of somatic origin the levels of Fraction I endorphins tend to be lower. It could be argued, that these low levels are a consequence of inadequate activity of endorphin systems and are therefore involved in genesis of the pain. However, in these patients, the pain was local and not the general phenomenon one might expect from an overall endorphin deficiency. It may be proposed that pain itself activates inhibitory mechanisms suppressing production of endorphins. Alternatively, something may cause a local deficiency in endorphins, resulting in local pain. At any rate, these observations are consonant with the hypothesis that endorphin activity after all may be modulated by environmental influences.

Practically nothing is known regarding possible specific roles of pituitary endorphins. Their greater metabolic stability suggests that they would have sustained effects. The existence of opiate receptors in the pituitary (56) might indicate that they act locally, perhaps influencing release of prolactin, growth hormone, or antidiuretic hormone. Available evidence suggests a direct action on neural tissue (57).

### *Stimulation-Produced Analgesia*

Slightly before the biochemical identification of endorphins it was reported that powerful analgesia could be elicited in experimental animals and in man by intracerebral electrical stimulation. Reynolds, in his pioneering work (3), showed that stimulation of areas within the brain stem with intracerebral electrodes gave surgical analgesia in rats. This work was confirmed and extended particularly by Akil, Liebeskind & Mayer in a series of publications (see 4). Electrical stimulation of the periaqueductal gray matter was found to produce a high level of analgesia in rats and cats. Although, judging from the stimulus parameters used, it is likely that widespread effects were produced, thereby limiting interpretation relative to possible mechanisms of action, several interesting conclusions have been reached. The analgesia is probably partly due to activation of descending fibers that inhibit spinal responses to noxious stimuli (58, 59). Some of these fibers may be serotonergic since decreased serotonergic tonus inhibits stimulation-produced analgesia (60). Descending substance P fibers may also be involved, since there is an intimate spatial relationship between substance P terminals and enkephalin fibers in the dorsal horn

**Table 1** Endorphin (Fraction I) levels in human lumbar CSF as measured in a receptor binding assay (34)[a]

| Subjects | n | Endorphin level (calculated as pmol/ml methionine-enkephalin) |
|---|---|---|
| Healthy volunteers | 19 | 1.04 ± 0.08 |
| Neurologic cases (nonpain) | 9 | 1.4 ± 0.4 |
| Chronic pain (somatic origin) | 11 | 0.34 ± 0.09 |

[a] Means ± SEM are given.

of the spinal cord (38). Furthermore, substance P is active as a naloxone-reversible analgesic (61). Since substance P lacks affinity for opiate receptors (62), this action is probably indirect, perhaps via activation of enkephalin fibers (61). It should be emphasized that interactions between substance P and the endorphins seem to be complex. Some pain afferents are probably substance P fibers (38). Enkephalin fibers may form axoaxonic synapses with these primary afferents, and upon activation, block impulse flow in pain pathways (38, 63). Support for an interaction between the release of substance P and endorphins derives from the fact that endorphins inhibit release of substance P from the spinal trigeminus nucleus in vitro (64). Electrically produced or induced analgesia is partly naloxone-reversible (65). It has also been found that morphine-tolerant animals respond less well to electrostimulation than a naive animal (66); that is, there appears to be cross tolerance suggesting that the stimulation ultimately acts by activating endorphin neurons.

Pain relief produced by intracerebral stimulation has been obtained in patients with severe chronic pain (67–69) using electrodes chronically implanted into sites adjacent to the third ventricle. Usually 15–45 minutes' stimulation is sufficient to induce analgesia of several hours duration. Very interestingly, Adams (67) and Meyerson et al (70) report that in some patients the analgesic response is antagonized by naloxone. Furthermore, in two of three patients where the analgesia was naloxone-reversible, lumbar CSF levels of endorphins rose after stimulation (70). Similar results were recently reported by Hughes (71). The evidence for an involvement of endorphins in electrically induced analgesia is therefore very strong (67, 70, 71).

Another approach to inducing analgesia with physical methods is that of acupuncture pioneered by the Chinese several thousand years ago (72). Scientific analysis of this method has demonstrated that it is valid and not dependent on hypnotic or distracting effects (73–74). The needles can be replaced by indwelling or surface electrodes which are stimulated electrically (75). Acupuncture-like electrostimulation produces naloxone-reversible analgesia (76). In some patients undergoing such analgesia, lumbar CSF endorphin levels increase. Evidence for a segmental activation of endorphins derives from the fact that this occurs only in patients who are stimulated via pathways involving lumbar segments of the spinal cord (77). The analgesia produced by classical acupuncture in man is also naloxone-reversible (78).

While intracerebral stimulation may produce analgesia over quite large areas of the body (4, 58), acupuncture and electroacupuncture produce a localized analgesic effect somewhat segmentally related to the stimulation site (73–74, 79), indicating that specific neuronal pathways are being activated. With this in mind it is difficult to understand the results of Pomeranz et al (80) who found that hypophysectomized rats did not respond to electroacupuncture as their normal controls did, and therefore advanced the hypothesis that pituitary endorphins are the mediators of analgesia. If this were the case one should expect a general analgesia from acupuncture. Several alternative explanations may be offered to explain the results of Pomeranz et al; for instance, hypophysectomy may alter the response to opioids (5).

There are also reports that electrostimulation of rat mesencephalon produces analgesia by mechanisms which are not naloxone-reversible (81, 82), and in the

work of Akil and associates only partial antagonism was achieved with naloxone (65). It is therefore probable that mechanisms other than endorphin activation may cause analgesia. Other mechanisms also seem to operate in conventional, high frequency transcutaneous electrostimulation in patients with chronic pain. Here pain relief is induced almost instantaneously in contrast to the very gradual onset of analgesia after acupuncture or electroacupuncture (75, 79, 83–84). Furthermore, analgesia produced by high frequency stimulation (85) or by hypnosis (86) is resistant to naloxone.

In conclusion, electrostimulation analgesia should not be regarded as a simple phenomenon. Depending on stimulation parameters and locus of stimulation, different mechanisms can operate. There seems to be little doubt, however, that endorphin systems contribute to the analgesia.



## PHARMACOLOGY OF ENDORPHINS

### *Acute Effects*

The simple structure of the enkephalins makes their synthesis easy. The number of analogues synthesized so far may be counted in the hundreds. Essentially two different approaches have been taken to evaluate structure-activity relationships: receptor binding assays or in vitro test systems such as the isolated mouse vas deferens or the guinea pig ileum. It has been found that the minimum active sequence is that of the enkephalin pentapeptides, methionine-enkephalin being about twice as potent as leucine-enkephalin. Deletion of the N-terminal tyrosine removes almost all activity. Other positions are also critical, and only small structural changes are tolerated [see further review by Frederickson (20)].

One consequence of these in vitro studies has been the demonstration of multiple opioid receptors. In addition to the receptor sites occupied by classical morphine-like opiates, there are sites with selectivity for opioid peptides (87–89). Furthermore, naloxone has less affinity for these peptide sites than against opioid alkaloid sites. Different tissues may have different populations of these receptors. Thus, the mouse vas deferens (87) and a neuroblastoma X glioma hybrid cell line (90) seem to possess mainly "peptide" receptor sites while the guinea pig ileum is richer in sites with high affinity for classical morphine-like compounds (87). It is not known whether regional differences in receptor populations exist in the CNS.

In neurophysiological studies the enkephalins seem to behave as morphine-like alkaloids. Recording single-cell activity in various regions of brain following microiontophoretic application of morphine or enkephalin shows depression or excitation. Only the former activity is usually reversible by naloxone and shows stereospecificity and is therefore thought to represent interaction with specific opioid receptors (91–93). In morphine-tolerant animals both morphine and the enkephalins show reduced activity (92). In these microinjection studies the potency of the enkephalins is comparable to that of morphine.

The electrophysiological data indicate that the enkephalins, like morphine, have a potent action in various brain areas. These observations suggest that the enkepha-

lins may be inhibitory neurotransmitters, which, like morphine, act by reducing the reaction to painful stimuli.

The naturally occurring enkephalins are very inactive as analgesics. High doses given intraventricularly or intracerebrally are required to produce a short-lasting analgesia in rats (94–95). Other workers have found it difficult to show any analgesia at all (96). Since the enkephalins have comparatively high affinity for opiate receptors in vitro (88–89, 97), this inactivity may depend on their kinetics and particularly on their vulnerability to metabolic degradation. Thus, considerable work has been directed to the synthesis of more active analogues. Analogues with D-alanine in position 2 (replacing Gly) retain the receptor affinity of enkephalin and are about as potent as morphine in producing analgesia after intracerebral administration. They are metabolically stable under in vitro conditions (96, 98, 99). Some of these analogues are also reasonably active after intravenous injection (98). The analgesia produced by all these peptides is readily reversed by naloxone.

Studies with the longer endorphins have focused largely on $\beta$-endorphin. This peptide is a very potent analgesic after intraventricular administration in cats (100) and rats (101–102), and is about 20 times more potent than morphine on a molar basis. In one study mentioned earlier, $\beta$-endorphin, given intravenously, produced analgesia in mice only when comparatively high doses were used (50–100 $\mu$g per animal) (27). The analgesia produced by these peptides was reduced within minutes by naloxone.

Responses other than analgesia have attracted much less attention and available data are only reviewed briefly here. Like morphine, high doses of $\beta$-endorphin causes catatonia in rats (103–104). $\beta$-Endorphin, like morphine, induces excessive grooming (105) and influences the sexual behavior of male rats (106). Reports are also available on a facilitatory action of endorphins on habituation (107). These observations (103–107) are interesting in the present context since pain and particularly the reactions to pain involve a number of brain centers and functions.

### *Chronic Effects*

The discovery of the endorphin peptides created hopes that these substances or analogues might provide the "ideal" analgesics with no abuse potential. The available evidence is not favorable. Enkephalin analogues have been reported to suppress abstinence reactions during withdrawal in morphine-addicted rats (108). Several studies indicate that opioid peptides induce tolerance and dependence of the morphine type. Wei & Loh (109) used an ingenious approach to the chronic delivery of opioid peptides. Small implantable micropumps,[1] driven osmotically at constant delivery rates, were connected to the lateral ventricles of conscious rats. The pumps were allowed to deliver morphine, methionine-enkephalin, or $\beta$-endorphin over 70 hr. When challenged with naloxone, animals in all groups responded with typical abstinence reactions (teeth chattering, wet dog shakes, diarrhea). The dose of $\beta$-endorphin needed to produce these effects was lower than needed for morphine or methionine-enkephalin by a factor of 10 (on molar basis). Bläsig & Herz (110) and

[1]Alzet®, Alza Corp., Palo Alto, California.

van Ree et al (111) also report both the development of tolerance to and dependence on opioid peptides and cross-tolerance to morphine on the part of animals tolerant to the analgesic action of the peptides.

Studies are presently being carried out on lower primates to assess the abuse potential of these peptides, but the results have not yet been published. No tests in humans have been reported. Until such information is available, the question regarding any clinical advantage of these peptides remains open to debate.

Like morphine, the enkephalins show positive reinforcement properties. Rats, participating in self-injection systems with access to the substance via permanent cannulae running into their lateral ventricles will inject leucine-enkephalin at a higher rate than morphine (112). Endorphins may therefore be endogenous euphoric agents. An interesting experiment by the same authors (112) lends further support to this hypothesis. Rats with permanent electrodes implanted in the pontine central gray (chosen because its stimulation induces analgesia as well as high rates of self-stimulation) were trained to self-stimulate. If naloxone was given to these animals, a dose-related decrease in the self-stimulation rate was observed. This and additional information were taken to suggest that endorphins are released on stimulation providing the animal a reward that reduces drive.

If the endorphins constitute endogeneous euphorigens or reward transmitters as suggested from the above-mentioned experiments, they can only be partly beneficial for the well-being of the individual. Prolonged states of analgesia or a permanent sense of satisfaction would ultimately be harmful. It may be recalled, for instance, that congenital insensitivity to pain is a serious condition (113). Thus the modulatory influence of endorphins must be strongly controlled by feedback mechanisms. Three essentially different regulatory steps seem possible: reduction of the availability of endorphins, a reduction of the receptor sensitivity to available endorphins or opiates, or production of physiological antagonists (113a). Evidence for the first possibility has in fact been observed in patients with chronic pain (see above, Table 1), while the second and third may be referred to as tolerance. Every experiment so far described shows that opioid peptides, like opiates, produce tolerance. Very little, if anything, is known about the mechanism of tolerance. It must be a naturally operating process of a very specific character. Biochemical studies have suggested the mechanism to be associated with an adaptive increase in adenyl cyclase activity compensating for the inhibitory influence of opioids on this enzyme (114–115). The reduced sensitivity of the receptor is likely to persist if the opioid is removed from the system (withdrawal). In this situation, the endorphin systems may no longer be able to exert their modulatory influence on transmitter release. It has been suggested that when this occurs, abstinence reactions may be generated (116). Certainly, several other hypothetical mechanisms are possible.

## CONCLUSIONS

The discovery of morphine-like peptides, the endorphins, provides valuable insight and the potential for obtaining equally valuable information on pain and the body's response thereto. The total functional role of the endorphins remains to be defined

but there is ample evidence to suggest that it is extensive. Various exogenous and endogenous mechanisms influence their activity, and although they appear to be involved with pain the actions of endorphins are certainly not confined to pain sensation. Their capacity to modulate other CNS and pituitary functions may be equally important.

The study of endorphin systems has been greatly facilitated by the existence of narcotic antagonists. Some caution regarding their specificity is appropriate, however, and antagonist-reversal is a necessary but not sufficient criterion for an endorphin-mediated process. Further, the pharmacology of the opioid antagonists, such as naloxone, must be reassessed to ascertain that the effect they produce in the absence of exogenous opioids is not a consequence of their own activity as an agonist. Detailed studies on endorphin turnover, release, and metabolism are strongly needed.

The pharmacologic characteristics of the endorphins seem to be very similar to those of classical narcotic analgesics. Ready availability of potent and systemically active synthetic analogues of the enkephalins will facilitate detailed pharmacologic evaluation. It will soon be learned whether or not they are superior to classic narcotic analgesics. Since endorphin-selective receptors exist in nervous tissue this may just be possible.



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*Literature Cited*

1. Gammon, G. D., Starr, I. 1941. Studies on the relief of pain by counter-irritation. *J. Clin. Invest.* 20:13–20
2. Kane, K., Taub, A. 1975. A history of local electrical analgesia. *Pain* 1:125–38
3. Reynolds, D. V. 1969. Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* 164: 444–45
4. Mayer, D. J., Price, D. D. 1976. Central nervous system mechanisms of analgesia. *Pain* 2:379–404
5. Murray, W. J., Miller, J. W. 1960. Potency differences of morphine-type agents by radiant heat and "cramping" analgesic assays providing evidence for a potentiating substance from the posterior pituitary gland. *J. Pharmacol. Exp. Ther.* 128:380–83
6. Collier, H. O. J. 1973. Pharmacological mechanisms of drug dependence. *Proc. 5th Int. Congr. Pharmacol.*, ed. J. Cochin, I:65–76
7. Pert, C. B., Snyder, S. H. 1973. Opiate receptor: Demonstration in nervous tissue. *Science* 179:1011–14
8. Simon, E. J., Hiller, J. M., Edelman, I. 1973. Stereospecific binding of the potent narcotic analgesic $^3$H-etorphine to rat-brain homogenate. *Proc. Natl. Acad. Sci. USA* 70:1947–49
9. Terenius, L. 1973. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol. Toxicol.* 32:317–20
10. Hughes, J. 1975. Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* 88:295–308
11. Terenius, L., Wahlström, A. 1975. Search for an endogenous ligand for the opiate receptor. *Acta Physiol. Scand.* 94:74–81
12. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., Morris, H. R. 1975. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258:577–79
13. Cox, B. M., Opheim, K. E., Teschemacher, H., Goldstein, A. 1976. A

peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. *Life Sci.* 16:1777–82
14. Simantov, R., Snyder, S. H. 1976. Morphine-like peptides in mammalian brain: Isolation, structure elucidation, and interactions with the opiate receptor. *Proc. Natl. Acad. Sci. USA* 73:2515–19
15. Guillemin, R., Ling, N., Burgus, R. 1976. Endorphines, peptides, d'origine hypothalamique et neurohypophysaire à activité morphinomimétique. Isolement et structure moléculaire de l'α-endorphine. *C. R. Acad. Sci. Paris* 282:783–85
16. Bradbury, A. F., Smyth, D. G., Snell, C. R. 1976. Lipotropin: Precursor to two biologically active peptides. *Biochem. Biophys. Res. Commun.* 69: 950–56
17. Goldstein, A. 1976. Opioid peptides (endorphins) in pituitary and brain. *Science* 193:1081–86
18. Hughes, J., Kosterlitz, H. W. 1977. Opioid peptides. *Br. Med. Bull.* 33: 157–61
19. Snyder, S. H., Simantov, R. 1977. The opiate receptor and opioid peptides. *J. Neurochem.* 28:13–20
20. Frederickson, R. C. A. 1977. Enkephalin pentapeptides—A review of current evidence for a physiological role in vertebrate neurotransmission. *Life Sci.* 21:23–42
21. Guillemin, R. 1977. Endorphins, brain peptides that act like opiates. *N. Engl. J. Med.* 1:226–27
22. Polak, J. M., Sullivan, S. N., Bloom, S. R., Facer, P., Pearse, A. G. E. 1977. Enkephalin-like immunoreactivity in the human gastrointestinal tract. *Lancet* 1:972–74
23. Li, C. H., Chung, D. 1976. Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc. Natl. Acad. Sci. USA* 73:1145–48
24. Lazarus, L. H., Ling, N., Guillemin, R. 1976. β-Lipotropin as a prohormone for the morphinomimetic peptides endorphins and enkephalins. *Proc. Natl. Acad. Sci. USA* 73:2156–59
25. Bloom, F., Battenberg, E., Rossier, J., Ling, N., Leppaluoto, J., Vargo, T. M., Guillemin, R. 1977. Endorphins are located in the intermediate and anterior lobes of the pituitary gland, not in the neurohypophysis. *Life Sci.* 20:43–47
26. Ross, M., Dingledine, R., Cox, B. M., Goldstein, A. 1977. Distribution of endorphins (peptides with morphine-like pharmacological activity) in pituitary. *Brain Res.* 124:523–32
27. Tseng, L. -F., Loh, H. H., Li, C. H. 1976. β-Endorphin as a potent analgesic by intravenous injection. *Nature* 263:239–40
28. Simantov, R., Kuhar, M. J., Pasternak, G. W., Snyder, S. H. 1976. The regional distribution of a morphine-like factor enkephalin in monkey brain. *Brain Res.* 106:189–97
29. Smith, T. W., Hughes, J., Kosterlitz, H. W., Sosa, R. P. 1976. Enkephalins: isolation, distribution and function. In *Opiates and Endogenous Opioid Peptides,* ed. H. W. Kosterlitz, pp. 57–62. Amsterdam: Elsevier & North Holland
30. Coons, A. H. 1958. Fluorescent antibody methods. In *General Cytochemical Methods,* ed. J. F. Danielli, pp. 399–422. New York: Academic
31. Elde, R., Hökfelt, T., Johansson, O., Terenius, L. 1976. Immunohistochemical studies using antibodies to leucine-enkephalin: Initial observations on the nervous system of the rat. *Neuroscience* 1:349–51
32. Hökfelt, T., Elde, R., Johansson, O., Terenius, L., Stein, L. 1977. The distribution of enkephalin immunoreactive cell bodies in the rat central nervous system. *Neurosci. Lett.* 5:23–31
33. Hökfelt, T., Terenius, L., Elde, R. 1977. Different distribution patterns of endorphin neurons using against leucine-enkephalin, methionine-enkephalin. In preparation
34. Terenius, L., Wahlström, A. 1975. Morphine-like ligand for opiate receptors in human CSF. *Life Sci.* 16:1759–64
35. Pert, C. B., Pert, A., Tallman, J. F. 1976. Isolation of a novel endogenous opiate analgesic from human blood. *Proc. Natl. Acad. Sci. USA* 73:2226–30
36. Wahlström, A., Johansson, L., Terenius, L. 1976. Characterization of endorphines (endogenous morphine-like factors) in human CSF and brain extracts. In *Opiates and Endogenous Opioid Peptides,* ed. H. W. Kosterlitz, pp 49–56. Amsterdam: Elsevier/North-Holland Biomed. Press
37. Terenius, L., Wahlström, A. 1977. Physiological and clinical relevance of endorphins. In *Centrally Acting Peptides,* ed J. Hughes. London: Macmillan. In press
38. Hökfelt, T., Ljungdahl, Å., Terenius, L., Elde, R., Nilsson, G. 1977. Immunohis-

tochemical analysis of peptide pathways possibly related to pain and analgesia: Enkephalin and substance P. *Proc. Natl. Acad. Sci. USA* 74:3081–85
39. Tsou, K., Jang, C. -S. 1964. Studies on the site of analgesic action of morphine by intracerebral microinjection. *Sci. Sin.* 8:1099–1109
40. Herz, A., Albus, L., Metyš, J., Schubert, P., Teschemacher, H. 1970. On the central sites for the antinociceptive action of morphine and fentanyl. *Neuropharmacology* 9:539–51
41. Yaksh, T. L., Yeung, J. C., Rudy, T. A. 1976. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: Observation of differential effects within the periaqeductal gray. *Brain Res.* 114:83–103
42. Yaksh, T. L., Rudy, T. A. 1976. Analgesia mediated by a direct spinal action of narcotics. *Science* 192: 1357–58
43. Martin, W. R. 1967. Opioid antagonists. *Pharmacol. Rev.* 19:463–521
44. Jaffé, J. H. 1975. Drug addiction and drug abuse. In *The Pharmacological Basis of Therapeutics*, ed. L. S. Goodman, A. Gilman, pp. 284–324. London: Macmillan
45. Jacob, J. J., Tremblay, E. C., Colombel, M.-C. 1974. Facilitation de réactions nociceptives par la naloxone chez la souris et chez le rat. *Psychopharmacologia* 37:217–23
46. Walker, J. M., Berntson, G. G., Sandman, C. A., Coy, D. H., Schally, A. V., Kastin, A. J. 1977. An analog of enkephalin having prolonged opiate-like effects in vivo. *Science* 196:85–87
47. Goldstein, A., Pryor, G. T., Otis, L. S., Larsen, F. 1976. On the role of endogenous opioid peptides: Failure of naloxone to influence shock escape threshold in the rat. *Life Sci.* 18:599–604
48. Grevert, P., Goldstein, A. 1977. Effects of naloxone on experimentally induced ischemic pain and on mood in human subjects. *Proc. Natl. Acad. Sci. USA* 74:1291–94
49. El-Sobky, A., Dostrovsky, J. O., Wall, P. D. 1976. Lack of effect of naloxone on pain perception in humans. *Nature* 263:783–84
50. Gritz, E. R., Shiffman, S. M., Jarvik, M. E., Schlesinger, J., Charuvastra, V. C. 1976. Naltrexone: Physiological and psychological effects of single doses. *Clin. Pharmacol. Ther.* 19:773–76
51. Gunne, L.-M., Lindström, L., Terenius, L. 1977. Naloxone-induced reversal of schizophrenic hallucinations. *J. Neural Transm.* 40:13–19
52. Goldfarb, J., Hu, J. W. 1976. Enhancement of reflexes by naloxone in spinal cats. *Neuropharmacology* 15:785–92
53. Lal, H., Miksic, S., Smith, N. 1976. Naloxone antagonism of conditioned hyperthermia: An evidence for release of endogenous opioid. *Life Sci.* 18: 971–76
54. Melzack, R. 1973. *The Puzzle of Pain.* New York: Basic Books
55. Madden, J. IV, Akil, H., Patrick, R. L., Barchas, J. D. 1977. Stress-induced parallel changes in opioid levels and pain responsiveness in the rat. *Nature* 265:358–60
56. Simantov, R., Snyder, S. H. 1977. Opiate receptor binding in the pituitary gland. *Brain Res.* 124:178–84
57. Rivier, C., Vale, W., Ling, N., Brown, M., Guillemin, R. 1977. Stimulation *in vivo* of the secretion of prolactin and growth hormone by β-endorphin. *Endocrinology* 100:238–41
58. Oliveras, J. L., Besson, J. M., Guilbaud, G., Liebeskind, J. C. 1974. Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. *Exp. Brain Res.* 20:32–44
59. Basbaum, A. I., Clanton, C. H., Fields, H. L. 1976. Opiate and stimulus-produced analgesia: Functional anatomy of a medullospinal pathway. *Proc. Natl. Acad. Aci. USA* 73:4685–88
60. Akil, H., Liebeskind, J. C. 1975. Monoaminergic mechanisms of stimulation-produced analgesia. *Brain Res.* 94:279–96
61. Stewart, J. M., Getto, C. J., Neldner, K., Reeve, E. B., Krivoy, W. A., Zimmermann, E. 1976. Substance P and analgesia. *Nature* 262:784–85
62. Terenius, L. 1975. Effect of peptides and amino acids on dihydromorphine binding to the opiate receptor. *J. Pharm. Pharmacol.* 27:450–52
63. LaMotte, C., Pert, C. B., Snyder, S. H. 1976. Opiate receptor binding in primate spinal cord: Distribution and changes after dorsal root section. *Brain Res.* 112:407–12
64. Iversen, L. 1977. Distribution and release of substance P in rat brain. See Ref. 37
65. Akil, H., Mayer, D. J., Liebeskind, J. C. 1976. Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. *Science* 191:961–62

66. Mayer, D. J., Hayes, R. L. 1975. Stimulation-produced analgesia: Development of tolerance and cross-tolerance to morphine. *Science* 188:941–43
67. Adams, J. E. 1976. Naloxone reversal of analgesia produced by brain stimulation in the human. *Pain* 2:161–66
68. Gybels, J., Cosyns, P. 1976. Modulation of experimentally produced pain in man by electrical stimulation of some cortical, thalamic and basal ganglia structures. In *Sensory Functions of the Skin in Primates, with Special Reference to Man,* ed. Y. Zotterman, pp. 521–30. Oxford: Pergamon
69. Richardson, D. E., Akil, H. 1977. Pain reduction by electrical brain stimulation in man. *J. Neurosurg.* 47:178–83
70. Meyerson, B. J., Boethius, J., Terenius, L., Wahlström, A. 1977. Endorphin mechanisms in pain relief with intracerebral and dorsal column stimulation. *3rd Meet. Eur. Soc. Stereotactic Funct. Neurosurg.,* Freiburg, Sept. 19–21 (Abstr.)
71. Hughes, J. 1977. Biochemistry and pharmacology of enkephalins. See Ref. 37
72. Needham, J. 1977. *Celestial Lancets; a History and Rationale of Acupunture and Moxa.* Cambridge: Cambridge Univ. Press
73. Chiang, C.-Y., Chang, C.-T., Chu, H.-L., Yang, L.-F. 1973. Peripheral afferent pathway for acupuncture analgesia. *Sci. Sin.* 16:210–17
74. Stewart, D., Thomson, J., Oswald, I. 1977. Acupuncture analgesia: An experimental investigation. *Br. Med. J.* 1:67–70
75. Andersson, S. A., Ericson, T., Holmgren, E., Lindqvist, G. 1973. Electroacupuncture. Effect on pain threshold measured with electrical stimulation of teeth. *Brain Res.* 63:393–96
76. Sjölund, B., Eriksson, M. 1976. Electroacupuncture and endogenous morphines. *Lancet* 2:1085
77. Sjölund, B., Terenius, L., Eriksson, M. 1977. Increased cerebrospinal fluid levels of endorphins after electro-acupuncture. *Acta Physiol. Scand.* 100:382–84
78. Mayer, D. J., Price, D. D., Rafii, A. 1977. Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. *Brain Res.* 121:368–72
79. Eriksson, M., Sjölund, B. 1976. Acupuncturelike electroanalgesia in TNS-resistant chronic pain. In *Sensory Functions of the Skin,* ed. Y. Zotterman, pp. 575–81. Oxford & New York: Pergamon
80. Pomeranz, B., Cheng, R., Law, P. 1977. Acupuncture reduces electrophysiological and behavioral responses to noxious stimuli: Pituitary is implicated. *Exp. Neurol.* 54:172–78
81. Pert, A., Walter, M. 1976. Comparison between naloxone reversal of morphine and electrical stimulation induced analgesia in the rat mesencephalon. *Life Sci.* 19:1023–32
82. Yaksh, T. L., Yeung, J. C., Rudy, T. A. 1976. An inability to antagonize with naloxone the elevated nociceptive thresholds resulting from electrical stimulation of the mesencephalic central gray. *Life Sci.* 18:1193–98
83. Shealy, C. N., Maurer, D. 1974. Clinical use of conventional TNS. *Surg. Neurol.* 2:45–47
84. Melzack, R. 1975. Prolonged relief of pain by brief, intense transcutaneous somatic stimulation. *Pain* 1:357–73
85. Sjölund, B., Eriksson, M. 1977. Personal communication
86. Goldstein, A., Hilgard, E. R. 1975. Failure of the opiate antagonist naloxone to modify hypnotic analgesia. *Proc. Natl. Acad. Sci. USA* 72:2041–43
87. Lord, J. A. M., Waterfield, A. A., Hughes, J., Kosterlitz, H. W. 1977. Endogenous opioid peptides: Multiple agonists and receptors. *Nature* 267:495–99
88. Simantov, R., Snyder, S. H. 1976. Morphine-like peptides, leucine enkephalin and methionine enkephalin: Interactions with the opiate receptor. *Mol. Pharmacol.* 12:987–98
89. Terenius, L. 1977. Opioid peptides and opiates differ in receptor selectivity. *Psychoneuroendocrinology* 2:53–58
90. Wahlström, A., Brandt, M., Moroder, L., Wünsch, E., Lindeberg, G., Ragnarsson, U., Terenius, L., Hamprecht, B. 1977. Peptides related to β-lipotropin with opioid activity. *FEBS Lett.* 77:28–32
91. Bradley, P. B., Briggs, I., Gayton, R. J., Lambert, L. A. 1976. Effects of microiontophoretically applied methionine-enkephalin on single neurones in rat brainstem. *Nature* 261:425–26
92. Zieglgänsberger, W., Fry, J. P., Herz, A., Moroder, L., Wünsch, E. 1976. Enkephalin-induced inhibition of cortical neurones and the lack of this effect in morphine tolerant/dependent rats. *Brain Res.* 115:160–64
93. Frederickson, R. C. A., Norris, F. H. 1976. Enkephalin-induced depression

of single neurons in brain areas with opiate receptors—antagonism by naloxone. *Science* 194:440–42

94. Büscher, H. H., Hill, R. C., Römer, D., Cardinaux, F., Closse, A., Hauser, D., Pless, J. 1976. Evidence for analgesic activity of enkephalin in the mouse. *Nature* 261:423–25
95. Belluzzi, J. D., Grant, N., Garsky, V., Sarantakis, D., Wise, C. D., Stein, L. 1976. Analgesia induced in vivo by central administration of enkephalin in rat. *Nature* 260:625–26
96. Pert, C. B., Pert, A., Chang, J.-K., Fong, B. T. W. 1976. [D-Ala²]-Met-Enkephalinamide: A potent, long-lasting synthetic pentapeptide analgesic. *Science* 194:330–32
97. Bradbury, A. F., Smyth, D. G., Snell, C. R. 1976. C fragment of lipotropin has a high affinity for brain opiate receptors. *Nature* 260:793–95
98. Bajusz, S., Rónai, A. Z., Székely, J. I., Dunai-Kovács, Z., Berzétei, I., Gráf, L. 1976. Enkephalin analogs with enhanced opiate activity. *Acta Biochim. Biophys. Acad. Sci. Hung.* 11:305–9
99. Coy, D. H., Kastin, A. J., Schally, A. V., Morin, O., Caron, N. G., Labrie, F., Walker, J. M., Fertel, R., Berntson, G. G., Sandman, C. A. 1976. Synthesis and opioid activities of stereoisomers and other d-amino acid analogs of methionine-enkephalin. *Biochem. Biophys. Res. Commun.* 73:632–38
100. Feldberg, W., Smyth, D. G. 1976. Analgesia produced in cats by the C-fragment of lipotropin and by a synthetic pentapeptide. *J. Physiol.* 260: 30P–31P
101. Loh, H. H., Tseng, L. F., Wei, E., Li, C. H. 1976. β-Endorphin is a potent analgesic agent. *Proc. Natl. Acad. Sci. USA* 73:2895–98
102. Székely, J. I., Rónai, A. Z., Dunai-Kovács, Z., Gráf, L., Bajusz, S. 1976. C-terminal fragment (residues 61-91) of β-lipotropin: Is it the natural opiate-like neurohormon of the brain? *Experientia* 33:54–55
103. Bloom, F., Segal, D., Ling, N., Guillemin, R. 1976. Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* 194:630–32
104. Jacquet, Y. F., Marks, N. 1976. The C-fragment of β-lipotropin: An endogenous neuroleptic or antipsychotogen? *Science* 194:632–34
105. Gispen, W. H., Wiegant, V. M., Bradbury, A. F., Hulme, E. C., Smyth, D. G., Snell, C. R., de Wied, D. 1976. Induction of excessive grooming in the rat by fragments of lipotropin. *Nature* 264:794–95
106. Meyerson, B. J., Terenius, L. 1977. β-endorphin and male sexual behavior. *Eur. J. Pharmacol.* 42:191–92
107. de Wied, D. 1977. Behavioural effects of peptides related to β-lipotropin. See Ref. 37
108. Baxter, M. G., Follenfant, R. L., Miller, A. A., Sethna, D. M. 1977. Some morphine-like properties of a potent antinociceptive synthetic pentapeptide in relation to physical dependence in rodents. *Br. J. Pharmacol.* 59:523P
109. Wei, E., Loh, H. 1976. Physical dependence on opiate-like peptides. *Science* 193:1262–63
110. Bläsig, J., Herz, A. 1976. Tolerance and dependence induced by morphine-like pituitary peptides in rats. *Naunyn-Schmiedeberg's Arch. Pharmakol.* 294:297–300
111. van Ree, J. M., de Wied, D., Bradbury, A. F., Hulme, E. C., Smyth, D. G., Snell, C. R. 1976. Induction of tolerance to the analgesic action of lipotropin C-Fragment. *Nature* 264:792–94
112. Belluzzi, J. D., Stein, L. 1977. Enkephalin may mediate euphoria and drive-reduction reward. *Nature* 266:556–58
113. Thrush, D..C. 1973. Congenital insensitivity to pain. *Brain* 96:369–86

113a. Zimmermann, E., Krivoy, W. 1973. Antagonism between morphine and the polypeptides ACTH, ACTH 1-24, and β-MSH in the nervous system. *Prog. Brain Res.* 39:383–94

114. Brandt, M., Fischer, K., Moroder, L., Wiinsch, E., Hamprecht, B. 1976. Enkephalin evokes biochemical correlates of opiate tolerance and dependence in neuroblastoma X glioma hybrid cells. *FEBS Lett.* 68:38–40
115. Sharma, S. K., Klee, W. A., Nirenberg, M. 1975. Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. Natl. Acad. Sci. USA* 72:3092–96
116. Kosterlitz, H. W., Hughes, J. 1975. Some thoughts on the significance of enkephalin, the endogenous ligand. *Life Sci.* 17:91–96