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BIOLOGY OF OPIOID PEPTIDES

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INTRODUCTION

Opioid peptides are endogenous or synthetic peptides, with a spectrum of pharmacological activity similar to that of morphine and other narcotic agonist drugs. The designation opioid peptide can be made if (*a*) it produces morphine-like, naloxone reversible effects in several in vitro bioassay systems such as the guinea pig ileum (1), the cat nictitating membrane (2), the mouse vas deferens (3), and the neuroblastoma × glioma tissue culture preparation (4), and (*b*) if the peptide competes at low concentrations with opiate ligands for binding at opiate receptor binding sites (5–7). An additional, although not essential, criterion might include the production of naloxone reversible analgesia. These criteria exclude substance P which does have analgesic activity (8, 9), fragments of adrenocorticotropic hormone which have very weak opiate receptor binding activity (10), and the "morphine-like factor" identified on the basis of morphine antibody recognition (11).

At present only three endogenous opiate peptides have been identified that have any claim to a physiological function. These are leucine^5-enkephalin (12), methionine^5-enkephalin (12), and β-endorphin (13–16). Other fragments of β-lipotropin such as LPH 61–69, LPH 61–76, LPH 61–77, and LPH 61–87 have opioid peptide activity (17–19) but current evidence suggests that these may be artifacts of extraction or degradation products of β-endorphin (20). Other unidentified opioid peptides undoubtedly exist (21–24), but in the absence of structural and chemical identification it is impossible to assess their significance or biological function. The term *endorphin* as a generic name for opioid peptides has little to recommend

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it in our opinion and may actually create confusion; we have therefore avoided its use.

Research in this area has become so intense and wide ranging that an exhaustive coverage of the topic is beyond the scope of a single review. We concentrate on only a few selected areas of current interest and refer the reader to other recent reviews in this field (25–27).

EXTRACTION AND ASSAY OF ENDOGENOUS OPIOID PEPTIDES

The original extraction techniques were developed either for their convenience in handling large quantities of tissue, or for the ease of assaying the final product. There is no doubt that certain precautions must be taken in devising methods for the quantitative extraction and assay of β-endorphin and the enkephalins. These are very hydrophobic peptides and to avoid losses through adsorption it is necessary to use plastic or siliconized glassware; the addition of bovine serum albumin to solutions may further reduce these losses. Microwave irradiation (28, 29) has been used to avoid losses due to proteolytic degradation, but this is probably not a major source of loss if the tissues are dissected and extracted rapidly in medium capable of preventing proteolytic activity. The two most widely used methods, which give 85–95% recoveries, are homogenization in ice cold 0.1 M hydrochloric acid or boiling the tissue in 1 M acetic acid for 15 min prior to homogenization at 4°C. Some loss of β-endorphin may occur with hydrochloric acid extraction and although 5% trichloroacetic acid effectively extracts enkephalins, it will also cause precipitation and loss of the larger β-endorphin. Size separation of opioid peptides, particularly important in the differentiation of β-lipotropin and β-endorphin immunoreactivity, can be carried out on Sephadex G-50 in 50% acetic acid (30) or on Biogel P-4 (31). The enkephalins can be adsorbed onto Amberlite XAD-2® (32) or Porapak-Q® (33) and be quantitatively eluted with methanol or acetone. This procedure gives a considerable purification and provides salt-free solutions for bioassay or immunoassay. Methionine- and leucine-enkephalin can be separated by thin layer chromatography on silica gel (32). Oxidation of methionine-enkephalin can cause losses of this peptide, and the addition of dithiothreitol is recommended to prevent this occurring during chromatography or evaporation. The use of high efficiency liquid cation exchange and reverse phase chromatography to separate opiate peptides has been described by Rubinstein et al (34, 35).

Without doubt, radioimmunoassay is the most convenient and sensitive assay for β-endorphin and the enkephalins. Problems may arise from cross-reaction between β-endorphin antibodies and β-lipotropin, but very specific

antisera have been produced to the two enkephalins. Immunoassay of as little as 10 fmol of enkephalin has been reported, with less than 1% cross-reactivity between the two pentapeptides (36). The opiate receptor binding assay is not quite as convenient or sensitive (1 pmol) but it is useful as a general screen for opioid peptide activity and will differentiate between β-endorphin and β-lipotropin. The mouse vas deferens has a sensitivity of 1–5 pmol to the enkephalins and like the opiate receptor assay may be used as general screen for opioid peptide activity.

DISTRIBUTION OF OPIOID PEPTIDES

Biochemical and histochemical studies appear to have resolved the controversy concerning the relative distribution and importance of the enkephalins and β-endorphin. However, some caution is still required in interpreting the data since several unidentified opioid peptides have been detected in brain extracts (22, 24, 37) and cerebrospinal fluid (23). The most intriguing opioid (measured by receptor binding, immunoassay, or bioassay) appears to be a ligand of molecular weight between 800–1400 daltons (22, 23); it remains to be seen whether this is a metabolite of β-endorphin, an enkephalin precursor, or a hormone or neurotransmitter in its own right.

Several groups (22, 28, 29) have produced evidence that the enkephalins are not extraction artifacts or metabolites, although it appears that α- and possibly γ-endorphin may be formed during tissue extraction (20). Subcellular fractionation studies have shown that the enkephalins are concentrated in the synaptosomal fraction of brain homogenates (38, 39), a finding consistent with the proposed neurotransmitter/neuromodulator role of enkephalin (40).

Immunocytochemical studies have shown the existence of separate enkephalin- and β-endorphin–containing areas in the rat brain (41, 42). There appears to be a single β-endorphin/lipotropin system, with cell bodies located in the arcuate nucleus and long axons innervating midbrain and limbic structures (42, 43, 48). In contrast, enkephalin-containing cell bodies and nerve processes are widely distributed throughout the brain and spinal cord (44–49). It is estimated that there may be more than twenty separate enkephalin cell groups (51). The immunocytochemical studies are essentially in agreement with biochemical studies on discrete brain areas (22, 29, 36, 41) with the highest concentrations occurring in globus pallidus, striatum, hypothalamus, midbrain, and brainstem nuclei and the dorsal horn of the spinal cord. It is noteworthy that many areas that are rich in the enkephalins correspond to areas closely associated with dopaminergic, noradrenergic, serotonergic, and substance P–containing neuronal systems. In general the enkephalins appear to be particularly concentrated in brain

areas concerned with sensory transmission, endocrine control, respiration, motor activity, and behavior. The distributions of opiate receptor binding sites (52, 53) and enkephalin immunoreactive fibers show a close parallelism, with some exceptions such as the globus pallidus, nucleus interpeduncularis, and nucleus septi lateralis, which have high enkephalin levels but few opiate receptors (49, 50).

Many of the enkephalin-containing neurons appear to be short interneurons, such as in the substantia gelatinosa of the spinal cord (49). One long enkephalin-containing nerve pathway has been described (54) running from the caudate-putamen to the globus pallidus.

There is a rich enkephalinergic innervation of the gastrointestinal tract in several species (22, 32, 44), and there is evidence that the enkephalins may also be contained in various autonomic nerves and ganglian cells (22) and secretory mucosal cells of the stomach (55).

Although studies on discrete brain areas have revealed marked variations in the ratio of methionine-enkephalin to leucine-enkephalin (22, 29, 36, 49), the immunocytochemical studies have failed to show any difference in the distribution of the two pentapeptides. Further studies are required to determine whether enkephalins are contained within the same neurons, but this seems a very real possibility and if true this would pose some difficult questions regarding the functional role of this arrangement.

HYPOTHALAMIC-PITUITARY MECHANISMS

Effects on Pituitary Hormone Release

Goldstein and co-workers (56) originally detected the presence of opioid peptides in the pituitary gland. The publication of the enkephalin sequence and its homology with β-lipotropin led investigators to test the biological activity of its terminal C-fragment, which had previously been detected by chemical means. The C-terminal fragment (LPH 61–91), now known as β-endorphin, proved to be a major constituent of the intermediate lobe and to possess potent opiate-like activity and, in particular, analgesic activity (13, 14, 57). Immunohistochemical studies show a dense staining for β-endorphin-containing cells in the pars intermedia, with a sparser distribution of immunoreactive cells in the adenohypophysis; the neurohypophysis appears devoid of such activity (58). It is now clear that there is a very close and complex relationship between the opioid peptides and hypothalamic mechanisms. Although there are insignificant amounts of enkephalin in the pituitary, the hypothalamus is richly endowed with enkephalin-containing neurons and cell bodies (44–51).

The effects of narcotic analgesics on pituitary hormone release are well documented (59) and include effects on antidiuretic hormone, growth hor-

mone (GH), prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These effects are mediated by specific opiate receptors and are readily blocked by naloxone. Thus morphine inhibits the preovulatory surge of LH and FSH in rats and prevents ovulation (60, 61); it also stimulates the release of antidiuretic hormone, GH, and prolactin.

Intravenous or intraventricular injection of β-endorphin stimulates the release of prolactin and GH (62–65). These effects are abolished by naloxone or naltrexone. On a molar basis β-endorphin appears to be some twenty times more potent than morphine in this respect. Similar results have been obtained with leu^5-enkephalin, (D-Ala^2, Met^5)-enkephalinamide, (D-Ala^2, D-Leu^5)-enkephalinamide, met^5-enkephalin, and (D-Ala^2, Met (O)^5-ol)-enkephalin (66–73). Only the protected enkephalin analogues have potencies approaching that of β-endorphin. It has also been reported that intraperitoneal injection of met^5-enkephalin increases prolactin and GH serum levels and decreases LH and TSH levels (69). A direct action of the opioid peptides on the pituitary was suggested on the basis of rat pituitary cell culture experiments (66), but other results are at variance with this finding, (62, 68). Substance P has been reported to potentiate the GH and prolactin-releasing effects of morphine and β-endorphin (65). The opiate receptor–mediated release of GH and prolactin does not appear to involve somatostatin (65). A number of the above studies provided evidence that the opioid peptides have a preferential effect on prolactin release, with GH release occurring at higher dose levels; however, (D-Ala^2, D-Leu^5)-enkephalinamide appears to differ in that GH release occurs at low dose levels (72). Interestingly, the same study reports that somatostatin blocks morphine-stimulated GH release but has no effect on release of GH evoked by (D-Ala^2, D-Leu^5)-enkephalinamide.

If, as seems likely, the prolactin and GH-releasing activities of the opioid peptides are mediated via the hypothalamus, then what is the likely mechanism of action? The opioid peptides appear to act mainly by inhibiting neuronal activity. The release of adenohypophysial hormones may thereafter be due to a disinhibitory effect exerted directly on release inhibitory factors, or via dopaminergic or serotonergic mechanisms, which are thought to control the releasing factors (74–76).

Intravenous injection of β-endorphin, leu^5-enkephalin, and (D-Ala^2, D-Leu^5)-enkephalinamide also evokes the release of antidiuretic hormone in rats and rabbits (77, 78). A similar effect was observed with the intracerebral injection of leu^5-enkephalin at dose levels one tenth of those needed by the intravenous route (78). These effects are blocked by naloxone which itself has no effect on urine elimination. Again the opioid peptides do not appear to act directly on the neurohypophysis but probably act on the magnocellular nuclei or their afferent input. Mata et al (79) found a decrease

in pituitary opioid peptide levels in dehydrated rats, with increased release of oxytoxin and vasopressin. The authors suggest a modulatory role for opioid peptides on the neurohypophysis but there is little evidence for this at present.

There is indirect evidence that the opioid peptides have a physiological role in controlling the tonic release of adenohypophysial hormones. Thus the narcotic antagonist naltrexone has been shown to inhibit prolactin release both in normal rats and those subjected to foot shock (63). Naltrexone had no effect on release from isolated pituitaries and the antagonist did not reduce serum prolactin levels, which had been increased by administration of haloperidol or reserpine. Further, the effect of naltrexone was not affected by hypothalamic deafferentation, strongly indicating a hypothalamic site of action. The authors suggest that naltrexone may act via the arcuate nucleus–median eminence dopaminergic pathway (63). It has been similarly shown that naloxone lowers prolactin and GH serum levels (68, 69, 80) and increases serum LH and FSH levels but has little effect on serum TSH levels (69). There is thus a remarkable correspondence between the effects of the opioid peptides and narcotic analgesics on pituitary hormone release. The obverse effects of narcotic antagonists under basal or stress-induced conditions of hormone release suggest that the endogenous opioid peptides may have an important physiological role in controlling the release of these hormones. The exact mechanism of this control remains to be elucidated, but in the case of prolactin it may be that stress-induced release of enkephalin or β-endorphin leads to inhibition of dopamine release, which in turn leads to a decreased secretion of prolactin release inhibiting factor, thus causing increased secretion of prolactin.

Physiological Significance of Pituitary β-Endorphin

The role of pituitary β-endorphin is far from clear. It is not the source of brain opiate peptides since brain levels are unaffected by hypophysectomy (81, 82). Rat pituitary endorphin content increases markedly with age, with a rapid rise occurring 5 to 10 weeks after birth, coinciding with the onset of sexual maturity (83). Endorphin is contained within secretory granules and is firmly bound (84, 85). Both β-endorphin and adrenocorticotrophin (ACTH) plasma levels increase in parallel after stressful stimuli such as foot-shock (86) or leg break (87). This association between β-endorphin and ACTH might well reflect the proposed common origin and biogenesis of the two peptides (see later). It will be of considerable interest to determine whether this close relationship extends to control of release by the same corticotrophin-releasing factor.

Although rat plasma levels of β-endorphin increase by as much as five-fold during stress, the absolute plasma levels are still several orders of

magnitude lower than those required to produce analgesia after intravenous injection (87). However foot-shock stress can elicit an analgesic response which is at least partially reversed by naloxone (88). It is possible that β-endorphin has an unknown peripheral physiological role, perhaps related to changes in adrenocortical function or intermediary metabolism during stress. β-endorphin could possibly have an intrapituitary function since this tissue does possess opiate receptors (89), which could be involved in the modulation of hormone synthesis or release. Alternatively β-endorphin may be released into the hypothalamic portal system and be retrogradely transported so that it could act on hypothalamic or other brain centers. In this respect, retrograde transport of pituitary hormones in the pituitary stalk vasculature has been recently demonstrated (90).

As with ACTH, there is a close link between β-endorphin and adrenocortical function. A marked and significant increase in the levels of pituitary β-endorphin and ACTH occurs after adrenalectomy (87), which can be depressed by dexamethasone (87). The potency of intravenous β-endorphin is markedly increased by adrenalectomy, and this effect is also suppressed by dexamethasone (91). This appears to be a peripheral effect since the intraventricular potency of β-endorphin is not altered by adrenalectomy. The same authors found that neither hypophysectomy nor adrenalectomy alters the threshold to aversive stimuli.

RECEPTOR CLASSIFICATION

Behavioral and neurophysiological observation on the chronic spinal dog (92, 93) and parallel assays on the mouse vas deferens and guinea pig ileum (94) indicate that the ketocyclazocines and some N-dimethylfurylbenzomorphans differ from classical narcotic agonists in their interaction with the opiate receptor. Indeed, it may be no longer appropriate to discuss opiate and opioid peptide interactions in terms of a single opiate receptor site. There is no doubt that the enkephalins and β-endorphin interact with what may be termed the classical opiate receptor and a number of studies have confirmed the opiate receptor binding activity of the opioid peptides (95–104). However, certain discrepancies in rank order potencies are apparent when opioid peptides are tested in several different bioassay and binding tests (95–97, 100, 101, 105). It has been argued by Kosterlitz and his colleagues (96) that nonparallelism in these assays indicates the existence of more than one opiate receptor for the opioid peptides. The enkephalins, the enkephalin extended fragments of β-lipotropin up to LPH 61–77, and D-Ala^2-enkephalin analogues are all more potent in the mouse vas deferens assay than in the guinea pig ileum (96, 98). In the binding assay the enkephalins are more potent in displacing labeled enkephalins than ^{3}H-

naloxone and other opiate ligands (96, 99–103). In contrast β-endorphin is equipotent in the two bioassays and shows no preference in displacing various opiate or enkephalin ligands (96, 102, 103).

Numerous caveats can be applied to the interpretation of these results, and indeed caution is required since there are technical problems in comparing different assays. The biological lability of the enkephalins requires that these assays be performed at low temperature or in the presence of enzyme inhibitors such as bacitracin, and it is not yet certain how this may distort comparisons between different ligands. Low temperature, for example, may alter ligand molecular conformation and induce changes in the system unrelated to the nature of the receptor. Miller et al (106) have used iodinated D-ala^2-enkephalin derivatives and found that the monoiodinated compounds retain a high affinity for opiate receptors and that these compounds have the advantage of metabolic stability. However, the use of enkephalin derivatives poses problems in relating the results to the receptor interaction of the parent compounds.

It is most unlikely that differences in biological lability can explain all of the marked differences seen in the different bioassay systems. The protected analogue N-methyl-methionine-enkephalinamide (96) is more potent in the guinea pig ileum and less potent in the mouse vas deferens compared to the parent enkephalin; also, although there is no change in the inhibition of ^{3}H-naloxone binding, the N-methyl derivative has only 10% of the activity of methionine-enkephalin in displacing ^{3}H-leucine-enkephalin binding. These changes are most unlikely to result from a decrease in the rate of metabolism, and it appears that N-methylation and amidation has altered the spectrum of opiate activity toward that of a typical morphine agonist. A similar but more pronounced shift toward a typical morphine spectrum of biological activity is obtained with Tyr-Gly-Gly-Phe-NH-$(CH_2)_2$-CH-$(CH_3)_2$ where again potency in the guinea pig ileum and the displacement of ^{3}H-naloxone binding is increased, while activity decreases in the mouse vas deferens and in the ^{3}H-Leu-enkephalin binding assay. It appears that the free carboxyl-group of the C-terminal amino acid plays a critical role in determining "enkephalin-like" activity (107). Substitution of Gly^2 by D-Ala^2 considerably reduces the rate of metabolism of the enkephalins (108) and the resulting analogues have equally increased potency in both mouse vas deferens and guinea pig ileum, while the ability to displace ^{3}H-leu-enkephalin binding is unchanged (96, 107). It is likely that the increased activity in the bioassays is due to decreased degradation.

On the basis of the above results it has been proposed that different populations of opiate receptors exist in the brain and peripheral tissues (96). Typical morphine agonists are more potent in the guinea pig ileum; they are equally well antagonized by naloxone in both the guinea pig ileum and

mouse vas deferens (K_e= 2 nM for naloxone) and are more effective in displacing ^{3}H-naloxone, ^{3}H-naltrexone, or ^{3}H-dihydromorphine than ^{3}H-leu-enkephalin. These receptors have been designated μ-receptors (95, 96). The enkephalins and some enkephalin analogues are thought to interact with δ-receptors. The δ-agonists are more potent in the mouse vas deferens and are not so readily antagonized by naloxone in this preparation as in the guinea pig ileum (Ke $\simeq$ 20 nM compared to 2 nM). The δ receptor agonists are also more effective in displacing ^{3}H-leucine-enkephalin than ^{3}H-naloxone or ^{3}H-naltrexone. It appears that the guinea pig ileum contains a greater proportion of μ-receptors while the mouse vas deferens contains mainly δ-receptors. The differences between the agonists are of course relative, and no truly selective μ- or δ-agonist or, more important, antagonist, has yet been described. The opioid peptide β-endorphin is perhaps unique in that it appears to interact equally well in all the test systems, and this may well reflect some special attribute for its carboxyl terminal, as suggested by Smyth et al (109). Interestingly, β-endorphin shows no change in binding affinity when tested in sodium-containing or sodium-deficient media (110). However, the significance of the so-called sodium shift (111) is far from clear with respect to the opioid peptides, as met-enkephalin has a sodium shift of less than 10 (97) which would place it in the partial agonist class according to Pert el al (111), yet both methionine-enkephalin and β-endorphin behave as pure agonists in biological test systems.

At present the subclassification of opiate receptors remains tentative and there is no reliable way to estimate the relative proportions of μ- and δ-receptors in the central nervous system. Studies on the neuroblastoma X glioma hybrid cell model system indicate that these cells contain a predominance of δ-receptors (101). Further studies on different strains of mice (112) also support the concept of mixed receptor populations. Thus the enkephalins are equally effective on vasa deferentia from T/O and C57/BL mice, while the potencies of β-endorphin and normorphine are only 0.5 and 0.17 respectively in C57/BL mice compared to the T/O strain. The C57/BL mouse vas deferens apparently contains even fewer μ-receptors than the T/O strain.

Numerous enkephalin analogues have been synthesized which have enhanced in vivo biological activity (103, 113–116). These analogues have been produced mainly through modifications to the second and fifth amino acid positions of enkephalin, and this has led to decreased metabolism and increased penetration of the CNS, even after oral ingestion (113, 114). It is of interest that analogues such as FK 33–824 (73, 114) have a pharmacological profile much more akin to a μ-receptor agonist (H. W. Kosterlitz, personal communication), which makes it unlikely that this particular approach will yield a novel, nondependence producing analgesic.

MODE OF ACTION

Despite the possible differences in the receptor specificity of opiate and opiate peptide action, there is a remarkable correlation between the pharmacology of these agents, suggesting a common underlying mechanism of action. Thus the opiates and opiate peptides inhibit acetylcholine and noradrenaline release at low nerve stimulation frequencies in the peripheral nervous system (98) and inhibit substance P (117), dopamine (118), acetylcholine (119), and noradrenaline (120) release in the central nervous system. A previous review (121) has discussed the types of neurons likely to be involved in the action of morphine. Cross-tolerance occurs between morphine and the opioid peptides both in vitro and in vivo (122).

The electrophysiological aspects of opiate and opioid peptide action have been reviewed in some detail (123). In general, activation of opiate receptors leads to an inhibition of spontaneous electrical firing (124–128), firing induced by glutamate (125), and nociceptive nerve stimulation (129–132). At two sites, the Renshaw cells (133) and hippocampal pyramidal cells (127), opioid peptides and opiates elicit excitatory responses. The excitation of hippocampal cells probably results from disinhibition of GABA input onto these cells (W. Zieglgansberger, personal communication), and it remains to be seen whether the Renshaw cell is an exception to the almost universal depressant activity of the opioids. The ability of opioids to depress the postsynaptic excitatory action of glutamate (125) indicates a postsynaptic site of action. However, receptor binding studies (134, 135) and the ability of the opioids to decrease neurotransmitter release argue also for a presynaptic site of action.

Morphine and methionine-enkephalin hyperpolarize neurons in the myenteric plexus of the guinea pig (136), and this raises the possibility that dendritic hyperpolarization could play a role in the opioid inhibition of postsynaptic depolarization. Recently it has been shown that enkephalin depresses the glutamate-evoked response of cultured mouse spinal neurons in a noncompetitive manner (137). This effect seemed independent of any other effect on membrane properties, and the authors surmise that this may represent a neuromodulatory effect, distinct from classical neurotransmitter action. A further analysis of opioid action based on intracellular studies of this type is urgently needed to clarify the mechanism of action.

BEHAVIORAL AND OTHER CENTRAL EFFECTS

The potent in vivo activity of β-endorphin has stimulated a large number of studies on the behavioral effects of opioid peptides, and a number of these results have been reviewed by de Wied (138). Low doses of the opioid

peptides delay extinction of pole-jumping avoidance behavior and facilitate passive behavior (139). In rats β-endorphin causes abnormal sexual behavior (140), excessive grooming (141), stimulation of food intake (142), wet dog shakes (141, 144), and a decrease in general motor activity (139, 143–145). All these effects appear to be blocked by naloxone, but de Wied (130, 146) has noted that some behavioral effects may involve receptor types other than those of the classical opiate receptor. In view of the potent effects of the opioid peptides on pituitary hormone release it may be premature to conclude that all of these behavioral manifestations are direct effects.

Several investigators (23, 143, 145) have postulated an involvement of endogenous opioid peptides in the etiology of mental illness. Certainly the bizzare effects of relatively small doses of these peptides provide one link in what may be a causal relationship, and Terenius & Wahlström (23) have provided evidence for an ameliorative effect of narcotic antagonists in certain psychiatric states, although this has yet to be confirmed.

The enkephalins and enkephalin analogues are potent hypotensive agents, and at least part of this action may be centrally mediated (139). β-endorphin has a hypothermic effect in rats while the shorter lipotropin sequence 61–77 (γ-endorphin) causes hyperthermia (143).

BIOGENESIS

Studies with peptide and neurohypophysial hormones (148, 149) have shown that they are derived from the proteolytic cleavage of larger, biologically inactive prohormones, which are formed by RNA-directed ribosomal synthesis. In a number of cases it has also been shown that release of the biologically active peptide from its precursor is effected by intracellular trypsin-like enzymes which act specifically at paired basic residues (148). The most detailed studies on the synthesis of neuronal peptides have been carried out on oxytocin and vasopressin (149). These neurohypophysical hormones are synthesized in the cell bodies in the supraoptic nucleus and are subsequently transported in storage vesicles to the nerve endings in the posterior pituitary. It has been shown that after injection of labeled amino acids into the supraoptic nucleus there is an initial synthesis of large precursors in the cell body, followed by the gradual appearance, after 1–2 hr, of oxytoxin and vasopressin. The conversion proceeds by way of intermediate size precursors and occurs, in part at least, in the secretory granules during axonal transport.

The pituitary protein, β-lipotropin (1–91), has been found to contain the sequences of β-endorphin (βLPH 61–91), α-endorphin (βLPH 61–76), γ-endorphin (βLPH 61–77), and met enkephalin (βLPH 61–65) as well as melanocyte-stimulating hormone (βLPH 41–58). While LPH has no opiate

activity itself, it has been suggested that it may be a precursor for the opioid peptides. Evidence that lipotropin is a prohormone for the endorphins is reasonably strong. Thus the contiguous peptides βLPH 1–38, βLPH 41–58, and βLPH 61–91 have been isolated from the pituitary (150). Incubating β-lipotropin with supernatant extracts of rat brain resulted in the generation of peptides with morphinometic activity (151), and pulse labeling experiments have shown that isolated cells of the bovine pars intermedia produce β lipotropin and equivalent amounts of γ-lipotropin (βLPH 1–58) and β-endorphin (152). It has also been noted that the proposed cleavage points are at sites of paired basic residues in the lipotropin molecule.

The situation may be even more complex than first envisaged with recent reports that mouse pituitary tumor cells produce a large molecular weight (31,000) protein, which contains the sequence of both β-lipotropin and adrenocorticotropic hormone (153–155). The presence of this protein, termed *pro-opiocortin* has also been reported in extracts of rat pituitary (156). These findings are in line with other reports that there is a parallel distribution of βLPH and ACTH; they appear to be present in the same secretory granules and are released from the pituitary in a parallel fashion.

As yet no corresponding precursors have been found containing the leu-enkephalin sequence. Reports that a leu-endorphin may be present in dialysates of schizophrenics (157) are interesting but have yet to be substantiated.

There is now direct evidence that the enkephalins are formed from the breakdown of a larger precursor (158, 159). This has come from studies following the incorporation of ^{3}H-tyrosine into the enkephalins in isolated preparations of the guinea pig myenteric plexus and slices of guinea pig striatum. In both preparations a linear incorporation of label was observed only after an initial lag period of 1–2 hr following incubation with the ^{3}H-tyrosine. This incorporation was abolished when inhibitors of protein synthesis such as cyclohexamide or puromycin were included during the labeling period. The inhibitors had little or no effect when added after the initial lag period, indicating a specific action on ribosomal protein synthesis. The rate of incorporation of label into met-enkephalin was more rapid than for leu-enkephalin, possibly reflecting the lower concentrations of the latter normally found in these tissue stores. It has been suggested that the lag period represents the time taken for the synthesis and conversion of a larger prohormone.

The relationship between β-lipotropin, the endorphins, and met-enkephalin is intriguing. First, immunohistochemical techniques have demonstrated that the enkephalins are found in separate neuronal systems, both in the brain and the pituitary (41, 42, 48). It is unlikely therefore that the larger peptides can be long-lived precursors. Similarly if pro-opiocortin is

an enkephalin precursor then it must also be short lived, as its presence in enkephalinergic neurons would have been detected by β-endorphin antibodies. All the available evidence, however, points to any precursor(s) having more than a transient existence. The possibility must be considered that pro-opiocortin may be rapidly cleaved to form an N-terminal extended precursor of met-enkephalin. If so the presence of this peptide may be detectable with antibodies raised to β-melanocyte-stimulating hormone. Alternatively, the fact that βLPH contains the met-enkephalin sequence may only be of evolutionary and not of biosynthetic significance. With this in mind it is interesting to note that Yang et al (24) have detected two peptides in the caudate nucleus that cross-react with met-enkephalin antibodies. They have a larger molecular weight than met-enkephalin, can be distinguished from α- and β-endorphin, and have been suggested as possible precursors for the enkephalins. Similarly Hughes et al (22) have detected the presence of larger opioid peptides which may correspond to those reported by Yang et al. Further progress in this area will depend on the identification of these putative precursors and the development of reliable methods for estimating the rate of synthesis and turnover of the enkephalins.

METABOLISM OF OPIOID PEPTIDES

All the information available at present indicates that the enkephalins and β-endorphins are inactivated by enzymatic hydrolysis. The primary step in the very rapid (160, 161) degradation of the enkephalins involves N-terminal cleavage of tyrosine to yield the inactive gly-gly-phe-met or leu tetrapeptide (160). This aminopeptidase activity is widely distributed in the brain (162, 163), the highest activity occurring in the supernatant, synaptosomal, and nuclear fractions (162). Studies with enzyme inhibitors (162) indicate a considerable enzyme heterogeneity. Aminopeptidase activity can be inhibited by leucyl-B-napthylamide, *p*-chloromercuribenzoate (162, 164), bacitracin (165), and also by a mixture of simple dipeptides (166).

β-endorphin is far more stable than the enkephalins (108), its conformation appearing to protect the N-terminal tyrosine from hydrolysis. In contrast LPH 61–76 and 61–77 are far more susceptible to aminopeptidases (167), and formation of these peptides is probably the first stage in β-endorphin catabolism. Gráf & Kenessey (168) have shown that specific cleavage of β-lipotropin by a papain-like enzyme occurs between leu-phe-(βLPH 77–78) on incubation with a crude pituitary homogenate. Smyth & Snell (30) found that when β-endorphin was incubated with slices of rat striatum the principal products were γ-endorphin and free tyrosine. The inclusion of bacitracin had no effect on the half-life of β-endorphin but

increased the size of the γ-endorphin peak obtained, at the expense of tyrosine. The formation of a small amount of met-enkephalin was also claimed, but the inclusion of bacitracin had little effect on the amounts recovered. This is surprising in view of the fact that met-enkephalin is even more susceptible to aminopeptidase activity than γ-endorphin.

ENKEPHALIN RELEASE AND TURNOVER

A number of workers have produced indirect evidence for the release and participation of endogenous opioid peptides in physiological processes. Thus narcotic antagonists enhance acetylcholine release in the guinea pig ileum (169), noradrenaline release in brain slices (120), and facilitate motor responses in the guinea pig ileum (170, 171). Naloxone also enhances spinal reflexes (172, 173) and lowers the pain threshold in animals (174) and man (175). The action of narcotic antagonists on pituitary hormone release (see above) is also consistent with a physiological role for the opioid peptides. The effects of the narcotic antagonist are not generally pronounced, and the need to carefully adjust experimental conditions may explain the several negative reports of naloxone action, particularly on pain responsiveness (176).

The direct release of enkephalins has been reported by several groups. A potassium-evoked, calcium-dependent release of enkephalin was demonstrated with superfused synaptosomal preparations of rabbit striatum (32, 166). Similar effects of potassium have been shown with slices of rat striatum (177) and globus pallidus (178). Iversen et al (178) used bacitracin to block enkephalin breakdown and found that 9.5 ± 1.8% of the total tissue content was released during a 6 min exposure to potassium. Simple dipeptides were similarly used by Henderson et al (166) to demonstrate the release of both leucine- and methionine-enkephalin by veratridine from superfused slices of guinea pig striatum. No evidence for an extracellular conversion of β-endorphin to methionine-enkephalin was found in the latter study, making it unlikely that the source of the enkephalin was due to neuronally released β-endorphin. There is also evidence that enkephalin-like peptides are released into the cerebrospinal fluid during focal brain stimulation for analgesia in patients (179).

Changes in tissue levels of enkephalin have been reported. Stress decreases enkephalin levels in the rat hypothalamus but not in other brain regions (180). Chronic or acute administration of morphine does not change whole brain enkephalin levels (181). However, chronic treatment with some antipsychotic drugs (182), or lithium (183), increases the enkephalin content of the neostriatum, globus palidus and nucleus accumbens of rats, but not of the hypothalamus or hindbrain. Cycloheximide has been used to

inhibit enkephalin synthesis in the guinea pig ileum and the decline in enkephalin content during electrical stimulation taken as an index of peptide release (184).

CONCLUDING REMARKS

The opioid peptides appear to create euphoria not only in experimental animals (185) but also indirectly in the investigators themselves and consequently speculation in this field has been somewhat premature or based on insufficient evidence. However, this has led to a fresh appraisal and new approaches to neurobiological problems. It is now clear that the enkephalins are contained in nerves, are synthesized locally via ribosomal processes, and are released upon neuronal excitation. The enkephalins have a profile of pharmacological activity consistent with an action on opiate receptors leading to an inhibition of some types of neuronal activity. The evidence involving β-endorphin in neuronal processes is less complete but the pharmacological profile of this peptide shows an even closer relationship to narcotic agonists than the enkephalins. Current evidence suggests that the enkephalins are involved in a short-term, tonic inhibition of neurotransmitter processes while β-endorphin may be released under more extreme circumstances of stress or injury and may act for prolonged periods.

The role of opioid peptides in analgesic processes is perhaps a little clearer; short-term effects can be possibly attributed to the enkephalins, and the histochemical, electrophysiological, and pharmacological (186) evidence for enkephalinergic modulation in the spinal cord-substantia gelatinosa is impressive. Interestingly, although the enkephalins are not potent analgesics when injected into the central gray, unlike morphine and β-endorphin, a very potent effect is observed on injection into the nucleus gigantocellularis of the rat oblongata (187). Whether this represents a difference in the receptor selectivity or in the enzyme activity of this area remains to be determined.

Considerable uncertainties still exist as to the mode of action of the opioid peptides. Schmitt et al (188) have presented a view of neuronal control based on local circuits employing low voltage electronic potentials and involving multiple dendritic interactions. This model may have considerable significance for the understanding of opioid peptide action, and the recent results of Barker et al (137) would neatly fit into this concept of neuromodulation. Also of significance for opiate peptide action are observations suggesting that substance P (189) and somatostatin (190) are contained in the same neurons as biogenic amines. Kosterlitz & Hughes (40) originally suggested this as a theoretical possibility for the enkephalins where they would act as local neuromodulatory agents either on the same

neuron or on adjacent neurons or their dendrites. Experimental support for this concept with respect to the enkephalins has been recently obtained by T. Hokfelt and colleagues (personal communication).

The further definition of opiate receptor types and opioid peptide action together with a more complete understanding of the mechanisms underlying the biogenesis and release of these peptides will no doubt have an important impact on pharmacology. In the meantime some investigators may agree with Keats as in his "Ode to a Nightingale":

My heart aches and a drowsy numbness pains
My sense; as though of hemlock I had drunk
Or emptied some dull opiate to the drains
One minute past, and Lethe-wards had sunk.

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