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THE ENDORPHINS: A Growing Family of Pharmacologically Pertinent Peptides

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INTRODUCTION

That nuclear-powered rocket of neuropharmacology known as endorphin research continues to threaten a meltdown situation. New findings have materialized continuously throughout the six years since the peptides were first identified, and for the decade after the stereospecific ligand binding sites for opiates in brain membranes were characterized in vitro [see (1–4) for early reviews]. The original and review material on this general topic has become so voluminous that no general survey is possible within the limited space of an Annual Reviews chapter. Therefore, the present coverage highlights some of the major questions that have been answered as the endorphin vehicle went through lift-off, and poses some of the new questions that emerged during the short, accelerating thrust towards a permanent orbit in the firmament of neurotransmitters. Several recent monographs and reviews are cited for reference to most "older" data, which in this area now means pre-1978.

These questions are addressed: 1. how many endorphins are there and how are they related metabolically, structurally, or functionally; 2. how do the various means of detecting opiate receptors relate to the morphology of the peptide neural circuits that have been mapped; 3. what are the cellular actions of the several endorphins in the central and peripheral nervous system. Lastly, as the biology and metabolism of endorphins have become more precisely defined, their pharmacological involvement has outgrown the constraints of analgesia, tolerance, and addiction by which the endogenous "morphine" peptides were originally judged. Their broader pharmacological potential for regulation of eating, drinking, and blood pressure is

briefly considered in the conclusion. Because very recent detailed reviews have just appeared regarding the roles of the endorphins in the physiology of pain (5–7), in the etiology or treatment of the major psychoses (8–10), and in the performance of learned behaviors (11, 12), these topics are not considered.

HOW MANY ENDORPHINS ARE THERE?

The superfamily endorphin includes all endogenous peptides whose sequences include an enkephalin pentapeptide (either Met^5 enkephalin or Leu^5 enkephalin; see Table 1) and share some common actions at presumptive opiate receptors as defined by naloxone antagonism (with all of the imperfections of that definition) (see 13). The evidence on which the family tree has been constructed requires a brief background.

Table 1 Peptides of the superfamily endorphin[a]

I.	Peptides of the *pro-opiamelanocortin* series	
	A. Opioid peptides	
	β-endorphin	YGGFMTSEKSQTPLVTLFKNAIIKNAH (KKGQ)
	α-endorphin	YGGFMTSEKSQTPLVT
	γ-endorphin	YGGFMTSEKSQTPLVTL
	B. Nonopioids	
	γMSH	... SYSMEHFRWGKPV
	βMSH	DSGPYKMEHFRWGSPPRD
	γMSH_3	....YVMGHFRWDRPGRRNGSSSSGVGGAAQ
II.	Enkephalins	
	Met^5-enkephalin	YGGFM
	Leu^5-enkephalin	YGGFL
	Met^5-Arg^6-Phe^7-enkephalin	YGGFMRF
III.	C-terminally extended enkephalins	
	Dynorphin	YGGFLRRIRPKLKWDNQ
	α-neo endorphin	YGGFLRKYPK
	β-neo endorphin	YGGFLRKYP
IV.	Others	
	Kyotorphin	YR
	Dermophin	YdAFGYPSNH_2
	Casei-Morphin	YPFPGPI

[a] Unless otherwise indicated, all N- and C-termini are unblocked; common sequences are underlined.

Currently conceived principles of peptide neurochemistry hold that the secreted form is cleaved from a larger precursor peptide during storage or secretion. A corollary concept is that if the larger form is also biologically active in its noncleaved form, then it deserves consideration as an independent agonist. A further corollary, which is by no means ironclad, is that potential cleavage sites within a propeptide are basic dipeptides (Arg or Lys). Two major problems are inherent in attempts to characterize endorphins based on bioassay of factors present in tissue extracts: (*a*) the peptides are generally present in only picomol amounts, requiring pooling of presumptive tissue sources; and (*b*) endogenous peptidases (see 14, 15) acting on the natural materials can destroy active species as well as generate them from inactive precursors. Recombinant DNA biochemistry has partially overcome these problems by permitting the sequence of the propeptide to be determined directly from the mRNA of endorphin-producing cells.

Based on what has been learned thus far from the classical approaches to neuropeptide characterizations and from the newer mRNA analysis paradigms, there are at least three major branches of the superfamily endorphin.

1. The Pro-opiomelanocortin (POMC) family (16–22) is expressed independently (for review see 23–25) in adenohypophysis, intermediate lobe, and a single cluster of CNS neurons (see 11, 23–25). The major endorphin agonist derived from the preprohormone, POMC, is beta-endorphin, the 31 amino acid C-terminal sequence of Beta-lipotropin (B-LPH) (for review see 23, 24). However, shorter cleavage products, alpha-endorphin, and gamma-(24) endorphin, were also purified, isolated, and sequenced on the basis of their opiate action in bioassay (25), and have been reported to be present in brain extracts, along with their N-terminally cleaved products (des-TYR alpha- and des-TYR gamma-endorphin) (26). The latter peptides are not opiate agonists, but do have other biological actions in some hands (cf 27–30). Whether or not these cleavage products should be accepted as nonopiate agonists for other regulatory actions remains unclear, especially since the conditions under which they were isolated did not seek to prevent artifactual exposure to a prevalent des-Tyr-peptidase (15). The POMC precursor is processed to different end products in the three cellular sources: to corticotropin and beta-LPH in adenohypophysis, to alpha melanocyte stimulating hormone (MSH) and beta-endorphin in intermediate lobe, and to alpha MSH, beta-endorphin, or acetylated, and therefore inactive, beta-endorphin derived forms in CNS (23, 24, 31). The "melano" part of the name POMC refers to the basic heptapeptide of α-MSH; a homologous sequence is also present in β-MSH, and was then found for the third time (γ-MSH), by cDNA methods in the N-terminal domain of POMC (16–18). The major naturally occurring γ-MSH is C-terminally extended from the

homologous peptide sequence, is biologically active, and has been detected in all three POMC tissue sources (see 20–22) (Table 1).

2. The second branch of the endorphin family is that of the prototypic enkephalins (1–4) in which the 6:1 tissue ratio of Met^5: Leu^5-enkephalin seems now to be accounted for by a large proenkephalin (32–34) containing this ratio of pentapeptides framed by basic dipeptide cleavage markers. As purified from the mRNAs of adrenal medulla or from chromaffin tissue tumors, rich sources of enkephalin peptides in some species (see 35, 36), this enkephalin prohormone is independent of the POMC branch of the family, and at least on the basis of this mRNA analysis of the autonomic organ sources (see 36), is also independent of the other branches of the family tree. This finding confirms earlier assay and mapping studies, which separated the enkephalin and beta-endorphin systems (see 8, 23, 24, 36, 37) on morphological evidence before their separate molecular precursors were known.

3. The third branch of the family consists of the C-terminally extended Leu^5-enkephalin peptides, dynorphin (38–43), alpha-neoendorphin (44–46), and beta-neoendorphin (47), all of which contain a N-terminal Leu^5-enkephalin sequence followed by two or more basic amino acids with further C-terminal sequence extensions of different lengths. All three are potent agonists in bioassays without cleavage to the pentapeptide. Mapping studies (cf 42, 46, 47) suggest that these longer Leu-enkephalins may coexist within certain CNS circuits originally thought to contain classical enkephalins (37, 48–50). The dynorphin and alpha-neoendorphin agonists may also share a similar class of receptors (42, 43, 51).

In addition to these three established branches of the family tree, a few orphan agonists remain of uncertain parentage. The C-terminal heptapeptide of the adrenal medullary polyproenkephalin is Met^5, Arg^6, Phe^7 enkephalin (32–34). This peptide is also an effective opioid agonist without cleavage (see 52), and shares its C-terminal tetrapeptide, Phe-Met-Arg-Phe, with a C-terminally amidated molluscan excitatory peptide, FMRFamide, (from its one letter symbol amino acid structure) (53). Antibodies raised against FMRFamide recognize a unique system of mammalian neurons unlike the enkephalin, dynorphin, or POMC series circuits (54, 55), and the tetrapeptide itself has neuronal actions (56). Such peptides may account for other invertebrate enkephalin-like peptides detected by immunocytochemistry (57–59). A unique distant cousin of these peptides is dermophin (60), purified from frog skin, in which a "natural" d-Ala^2 residue appears; dermorphin bears strong resemblance to the caseimorphin peptides (see 61) extracted from milk casein, which by definition are "exorphins" rather than endogenous morphines. Even further removed from the mainline of the family is the dipeptide enkephalin releaser, kyotorphin (62), and the as-yet

uncharacterized endorphin factor found in human cerebrospinal fluid (63). A distant ancestral form may be the POMC-like material characterized by immunoassays on extracts of unicellular organisms (64).

This impressive array of natural substances, all sharing some properties of opiate agonists, makes it difficult to recall that the idea of an endogenous morphine-like factor was once considered rank speculation. The genealogical table may include even more members when the prohormone forms of dynorphin, alpha-neoendorphin, and the mammalian FMRFamide have been determined.

NEURAL DISTRIBUTION OF ENDORPHINS

Using antisera developed independently in several laboratories against POMC-derived peptides or N-terminally conjugated enkephalins, a series of progressively refined reports have described their quantitative and cytological distribution in the nervous system and other tissues (see 19, 23, 24, 37).

Enkephalins

The estimates of enkephalin content by radioimmunoassay (RIA) agree generally with the recent quantitative regional and histological estimates of the distribution of the "opiate receptors" (see 3, 37, 65). Nevertheless (see below), the morphological distribution of the enkephalin and B-E systems does not always parallel precisely the distribution of opiate receptors.

Although there have been further detailed RIA studies in rat (66) and other species (67–69) the best guide to peptide distribution in brain has been derived from immunohistochemistry. All of the studies describing immunocytochemical distribution patterns largely agree on the location of nuclear groups exhibiting enkephalin-immunoreactive (ir) nerve terminals in untreated rats, and of the ir-perikarya in rats pretreated with colchicine (see 8, 24, 37, 48, 49, 70–72). Colchicine pretreatment presumably facilitates the localization of enkephalin-containing perikarya by disaggregating microtubules and depressing cellulofugal transport of stored peptide so that immunoreactive materials accumulate within the perikaryon and decrease in terminal fields (37, 72, 73). However, enkaphalin-ir perikarya in pigeon brain are visible without colchicine (74).

In colchicine-pretreated rats, enkephalin-ir cells are detected throughout the central nervous system [see (24, 37) for review of details]. Nerve terminal-like patterns of immunoreactivity confirmed in some cases by electron microscopy (75–78) are densest in the globus pallidus and central nucleus of the amygdala, and show generally heavy innervation in other selected terminal fields from midbrain to spinal cord. The cells and fibers which

show enkephalin-ir in cerebral and hippocampal cortex remain open to interpretation among different users of the same antisera (cf 37, 48, 49, 79). Enkephalin-ir nerve fibers specifically innervate the source nuclei of all three major monoamine systems, the substantia nigra, the locus coeruleus, and the raphe nuclei. The brain region with the lowest (ir) enkephalin levels is the cerebellum; however, even here, a few reactive elements have been reported (80).

Possible Enkephalin Circuits in CNS

Several separate enkephalin-containing circuits have been proposed from stereotaxic knife cuts, lesions, or retrograde tracing, but all await physiological testing. Enkephalin-reactive spinal interneurons innervate the dorsal horn (see 37), but long descending spinal connections also exist (81) that are independent of dynorphin circuits (82). The enkephalinergic innervation of the globus pallidus may arise from the reactive neurons of the caudate nucleus and putamen, but this short diffuse pathway remains unclearly resolved (see 83, 84). Neurons of the paraventricular and supraoptic nuclei provide the enkephalin reactive fibers to the neural lobe, in rat (85) and monkey (86), and cat [(87), but see (86)].

CNS β-endorphin Content

When the same brain regions are compared for β-endorphin and enkephalin content, the two classes of opioid peptides vary independently from region to region (8, 23, 24, 37). Globus pallidus, caudate nucleus, and more caudal brain stem structures that contain dense enkephalin reactivity have virtually no β-endorphin. Even without colchicine, β-E reactive perikarya are demonstrable within two adjacent clusters in the tuberal zone of the hypothalamus and extend from dorsolateral portions of the middle of the arcuate nucleus anterolaterally below the ventromedial nucleus, reaching almost to the lateral border of the hypothalamus. No other immunoreactive β-endorphin neurons are observed in adult animals, even after colchicine pretreatment. However, other ir-cells may exist transiently early in postnatal development (73, 88). Extensive processes can be traced away from the immunoreactive neurons, with mainly midline fibers extending into the anterior hypothalamic-preoptic area and along the dorsal midline of the thalamus into the brainstem. These fibers extend towards the locus coeruleus and the parabrachial nuclei, and reach at least as far caudally as the nucleus tractus solitarius. Specific nuclei in which there appears to be extensive arborization of β-E immunoreactive fibers are the periventricular, paraventricular, ventromedial, and dorsomedial nuclei of the hypothalamus, the

paraventricular nucleus of the thalamus, the medial amygdala, septum, and the bed nucleus of the stria terminalis (BNST). The dorsal raphe and locus coeruleus nuclei are both innervated, but substantia nigra receives only a few fibers

of the POMC precursor. Because CNS anatomy of γ-MSH shows variations in density of terminal field innervation from β-endorphin, there is the possibility of differential processing of the common precursor to different end products within the same neurons (see 22).

Morphological Distinctions between Endorphin and Enkephalin Neurons

The neurons containing β-endorphin and enkephalin have distinct morphological features that may be of functional significance. Enkephalin immunoreactive (ir) cells are in general small short axon cells [but see (81)] with fine axons, groups of which are widespread throughout the nervous system. POMC neurons comprise a single relatively homogeneous cell type, larger and fusiform with one elongated series of periventricular targets (see 25). Thus, it seems reasonable to include the POMC neuronal system as a component of the more general endocrine peptidergic network in the periventricular hypothalamus (see 37). The neuroanatomy and heterogeneous morphology of enkephalin neurons suggest that enkephalin may mediate synaptic events of significance to many diverse areas and functions of the nervous system.

Distribution of C-terminally Extended Leu-Enkephalins

Although much less is known about the detailed anatomical locations of these peptides, what has been reported indicates that they are independent of the POMC or Met-enkephalin neurons. Both regional content and immunohistochemical mapping (39, 41, 45, 46, 79, 82, 88) distinguish these systems. The dynorphin and alpha-neoendorphin-ir neurons suggest these peptides coexist, especially in the magnocellular circuits to the posterior pituitary (45, 46, 88). The dynorphin cells of the magnocellular hypothalamic nuclei may also coexist with the vasopressin-ir neurons (88), and may parallel a separate enkephalin-ir projection (52). The intrahippocampal mossy fiber pathway shows dynorphin and alpha-neoendorphin-ir (46, 49, 79), which is visualizable directly; this result may explain the difficulties and conflicts in previous reports of enkephalin-ir elements of the rat hippocampus (cf 48, 49). The generally weak enkephalin-ir of cerebral cortex (49), like that of hippocampus (48–50), may be interpreted retrospectively as cross-reactivity with dynorphin-containing cells. Thus, the independent but overlapping existence of enkephalin circuits and dynorphin-alpha-neoendorphin circuits in spinal cord, hypothalamus, hippocampus, and presum-

ably other regions will continue to be a source of confusion until some means are found to examine the functions of these systems separately.

RECEPTOR MAPS AND CIRCUIT MAPS

Several excellent recent reviews have examined in detail the basis for discrimination of the several classes of opiate receptors, those macromolecular sites at which opiate alkaloids or opioid peptides act on intact tissue preparations to produce a biological response. As reviewed by Chang & Cuatrecasas (90), multiple methods have been brought to bear on the characterization and localization of opiate receptor subtypes. In addition to the physiological responses of isolated organs, receptor subgroups have been characterized by whole animal behaviors (such as the chronic spinal-transected dog preparation employed by Martin and his colleagues) (see 91), and by discriminative behavioral responses of experimental animals (92). In addition, electrophysiological actions have been employed in vivo (93, 94) and in vitro (95, 96). Complementing studies based on functional responses are the ligand binding assays which have been applied to broken membrane preparations of central and peripheral tissues (see 2, 3), and which have more recently been applied to slide mounted sections of tissues for localization of the binding sites by autoradiography (97–99).

These various approaches fully support the concept that there are multiple opiate receptor subtypes, of which some (the mu, kappa, and delta sites) can be assessed by both functional response and binding assays. The sigma site (of which there may be even further divisions) (see 90, 91), which may not in the end be truly opiate (92), is assessed mainly by whole animal responses. Although the resolution of the microscopic autoradiographic methods would be adequate to relate the ligand binding sites to known opioid peptide pathways, the evidence obtained to date does not break down easily into patterns that reflect the anatomy of the systems.

In comparing the general immunocytochemical results with the distribution of the chemically-detected or autoradiographically-detected opiate binding sites, some major discrepancies remain unexplained (see 2, 3, 37, 66): (*a*) binding sites are very dense in caudate and putamen but not in globus pallidus where the heaviest fibers are seen; (*b*) in contrast, the caudate has very few immunoreactive fibers but does show ir-perikarya (see 75); (*c*) cerebral cortex, which has receptors if studied by binding (see 2, 3, 97–100) and electrophysiologic techniques (94), shows sparse fiber or cell immunoreactivity [but see (79)]; (*d*) although nerve cells and fibers are exceedingly dense in the central nucleus of the amygdala, receptors are distributed more or less evenly throughout the entire amygdaloid complex.

These discrepancies may in part be due to the fact that some of the immunocytochemistry of enkephalins may have included cross-reactions with the unrelated C-terminally extended Leu-enkephalin peptide circuits that can coexist in the same regions. The lack of precise matching between ligand binding and peptide circuits may also reflect that other relevant peptide circuits will ultimately be identified. A third possibility is that neurons may manifest opiate receptors more diffusely on their cell surface rather than just at the synaptic regions at which they are contacted by fibers of one or another opioid peptide system. Thus, as an example, monoamine neurons may also exhibit significant numbers of opiate binding sites on their distal axons to account for effects of systemic opiates on transmitter release and for ligand binding in the sectioned brain, even in areas without endorphin cells or fibers. An enkephalin action in cerebral cortex that is dependent on intact catecholamine axons has, in fact, been reported (101, 102); furthermore, ligand binding pattern changes after some CNS lesions are also compatible with presynaptic localizations (see 5, 99). Thus, although such "noninnervated" receptors might be brought into action by systemically administered drugs that can enter the CNS, their utility to the function of the endogenous peptide circuits remains to be established. Nevertheless, this is probably the mode of action of opioid substances in the in vitro organ assays such as the ileum or vas deferens, where the assay depends on the inhibition of release of the cholinergic or noradrenergic transmitters.

Thus far, the best one-to-one concordance between receptor subclasses and opioid peptide systems has come from the work on dynorphin. Here Goldstein & Chavkin (42) and others (43, 51) have demonstrated convincingly that this peptide appears to act primarily as a kappa agonist, both centrally and in the guinea pig ileum preparation. Alpha-neoendorphin may have similar selective actions (43, 51). If these findings could be taken to exclude the kappa sites from receptors for beta-endorphin or the cleavage products of the proenkephalin series (which would include met^5-enkephalin, leu^5-enkephalin, and the Met enkephalin arg^6-phe^7) then these two peptide groups might be considered to share the mu and delta sites, in a manner similar to that by which noradrenergic circuits may use alpha and beta receptors on the same target cell. Such target cells bearing coexisting receptor subclasses may be difficult to examine electrophysiologically, especially in the absence of selective antagonists. Naloxone, the workhorse antagonist, has actions with different potencies at mu, delta, and kappa sites (see 13, 90, 91). However, the use of selective synthetic agonists, with ligand displacing properties that are selective among the subclasses of receptors, may most directly permit cellular responses associated with these receptors to be discriminated (cf, for example 90, 94, 103) and related to the specific neural circuitry from which the peptide agonists must be released.

In addition to the opiate actions of endorphins, other potent effects of beta-endorphin and its related POMC cleavage products have been observed on a variety of organ systems (104, 105), particularly the white blood cells (106–108), where the functional receptor appears to recognize the nonenkephalin C-terminus of the endorphin peptide, and the effects are not antagonized by naloxone. It is tempting to connect these latter effects with possible links between pituitary and immune system function (see 106–108).

Cellular Electrophysiology of Endorphins

Given the inability to separate receptor subclasses absolutely according to circuits, peptides, or receptor mechanisms, it is surprising that there is thus far relatively good agreement among the large number of electrophysiological studies testing endorphins or their synthetic analogs (for review see 24, 37, 93, 96, 109).

The predominant effect on neurons of virtually all brain regions is one of depression of spontaneous activity (24, 37, 109) associated in some cases with a hyperpolarization and an increased conductance of the membrane (111–113). These latter membrane effects occur with some depressant actions both on sympathetic and enteric neurons (112, 113) and on central noradrenergic neurons (111), but definitely not with all of those central neurons whose activity is depressed (95, 96, 110, 114). The inhibitory effects of opioid peptides on transmembrane properties of spinal neurons, either in vitro (114) or in vivo (115), are reported to occur without change in membrane potential or membrane resistance. In these cases, the inhibitory effects, detected as loss of excitation by amino acids or orthodromic inputs, has been attributed to a special postsynaptic action in which occupation of the opioid peptide receptor prevents activation of the ion channels to which excitatory transmitter is coupled. However (see 95, 96, 109, 110), it is uncertain whether such effects might not, in part, also be presynaptically mediated (altering excitatory transmitter release or depressing the excitability of excitatory interneurons). Both mechanisms could operate, since the antiglutamate effects of opiates, seen on some cells but not all (for review see 109), would speak to a postsynaptic site. I view this category of action as "disenabling" since opioid and other peptides frequently prevent responses of target neurons to other afferent transmitters; this general category of response complements the "enabling" effects of some monoamine transmitters (see 37).

In contrast to the depressant effects seen in most brain regions and all peripheral target cells, there are three groups of neurons that give excitatory responses, which are stereo-specific and naloxone-reversible: hippocampal pyramidal neurons (24, 37, 109), Renshaw interneurons (24, 37, 116), and

neurons of the lateral reticular nucleus (37, 109, 117). Because the hippocampus excitatory response has been pursued the most extensively in vivo and in vitro, and because this action could underlie important behavioral and neurological consequences (see 110, 118) it deserves separate consideration.

Endorphins and the Hippocampus

Early immunohistochemical investigations of enkephalin distribution revealed sparse labeling of cells or neuropil in the hippocampus (cf 48, 49), as did radioimmunoassays (24, 37, 73, 110). More recent immunocytochemical studies partially revealed significant amounts of enkephalin (IR) in the dentate-CA4-CA3 cellular fields with only half as much ENK-IR in CA1 and the subiculum (110, 119; see also 46, 73, 79). However, immunohistochemical studies with antisera raised against dynorphin (DYN) or leu-enkephalin both demonstrate IR in rat, cat, and squirrel monkey hippocampal mossy fibers, and in their source neurons, the dentate granule cells (see 79, 119). In addition, all hippocampal fields exhibit scattered small DYN (IR) and ENK (IR) cells (49, 73, 79). Present data suggest that there may be three sources of opioid peptides in rodent hippocampus: (*a*) intrinsic enkephalin or dynorphin interneurons; (*b*) a dentate-CA3 mossy fiber system containing dynorphin and α-neoendorphin; (*c*) an exogenous projection from entorhinal cortex containing enkephalin reactive axons (49, 79).

Several major questions persist as to the definitive mechanism of action of opioids in the hippocampus, in spite of the several reports on this subject. The original hypothesis (120), that morphine, endorphins, and enkephalins excite CA1 pyramidal cells via a disinhibitory mechanism (inhibiting neighboring inhibitory interneurons), is well supported (see 37, 109, 110, 118). Thus, whereas the intracellular studies by ourselves (95, 110) and others (96, 109, 121) on CA1 pyramidal cells of the hippocampal slice generally show an opioid-induced decrease in the IPSP size, with little direct change in membrane potential or resistance, there are exceptions (see 109, 110). Furthermore, there is a naloxone-sensitive suppression of depolarizing responses to glutamate by D-ala^2-D-leu^5 enkephalin (DADL) in CA1 pyramidal cells, but not by D-ala^2 met^5 enkephalin amide (DAMEA) (110). The source of these discrepancies is not clear, although the choice of synthetic or endogenous opioid peptide agonist and the receptor occupied may be critical. Direct analysis of a peptide releasing CNS circuit is awaited.

In preliminary studies on dynorphin and other kappa agonists, the majority of cells studied also exhibit naloxone-reversible excitatory responses (119). Excitatory effects of DYN, DYN 1–13, ethylketocyclazocine, α-neoendorphin and DYN 1–8 (in rough order of potency), which were blocked by naloxone, may also suggest an indirect mechanism for these

effects, generalized to a mu or delta type receptor. The key open issue is whether naloxone-resistant inhibitory effects (see 119) are indicative of kappa receptor actions or merely nonspecific.

More intriguing is the role of dynorphin (IR) and α-neoendorphin (IR) observed in the mossy fiber system—the acknowledged primary intrinsic input to CA3–4 hippocampal pyramidal neurons. Classically, the mossy fiber terminals have been thought to synapse directly on the proximal dendrites of CA3–4 neurons and to release an excitatory amino acid, as yet uncharacterized (121). It remains unclear what specific role(s) a coexisting peptide, e.g. (dynorphin or α-neoendorphin or both) may have in this major intrinsic fiber system. A simple suggestion in keeping with the properties of other opioid peptides is that the peptide cotransmitter would act to terminate (or disenable) the excitatory transmitter to sharpen the time resolution of the synaptic message, but at sites unique from other opioid receptors existing elsewhere in the hippocampal formation. The non-naloxone-reversible inhibitory responses observed in CA3–CA4 hippocampal neurons may reflect this effect.

CONCLUSIONS

Initially, the pharmacologic horizons of the endorphins were framed by the desire to determine their relationship to opiate analgesia (see 1–7) and opiate addiction. While there has been some recent success in defining the nature of the receptor adaptation that occurs during the development of opiate tolerance (see 122–125; see also 90–94), this remains an open issue in need of methods that can dissociate the phenomena of molecular and cellular tolerance from those of the whole behaving animal (cf 92, 93). Behavioral tolerance has not yet been attributed to a change in ligand binding or endogenous peptide content.

With no methods yet available to determine synthesis rates of the endorphins [but see (19) for a beginning], functional evidence of their participation in the effects of other psychoactive drugs has generally been restricted to changes in content (126–132) or in the ability of exogenous peptides or opiates to alter the turnover rates of monoamines, of which the dopaminergic and cholinergic systems appear most responsive (133, 134).

Drug development efforts to obtain therapeutic agents that can modify endorphin function have taken two main avenues: (*a*) synthetic peptide agonists with potency as analgesics (see 4–6, 90), and (*b*) inhibitors of the enzymes that can break down the active agonist forms of enkephalin (14) and thus improve analgesia potency of the endorphin species released endogenously. There is some indication that exogenous opioid peptides can serve as competitive substrates for endogenous peptides (135) and that novel

species of enkephalinases may be amenable to selective antagonism (see 14, 15).

Such efforts have so far neglected at least two other important avenues in which endorphin drug development is sorely needed: (*a*) selective antagonists capable of discriminating either among central and peripheral receptors [see (136, 137) for a beginning], or among the subclasses of opiate receptors; (*b*) drugs that could activate or antagonize the special sets of receptors that may help clarify the roles of endorphins (central or peripheral) in such essential vegetative actions as blood pressure (138, 139) and flow (140), core temperature (for review see 141), and food (142, 143) or water intake (144). A large and fiercely contested literature surrounds the actions of this latter pharmacology. When "endorphins" are defined only as endogenous substances whose effects are revealed by naloxone blockade, they have been alleged to be involved in clinical phenomena ranging from alcoholic stupor (145) to exercise tolerance (146).

Central to the ultimate interpretation of these potential involvements is the issue of whether plasma-borne endorphins are "functional" as judged by opiate receptor pharmacology. This question has potential answers at two levels of currently unanswerable complexity: 1. Blood-borne endorphins either do (147, 148) or do not (149, 150) enter the CNS. If they do, then a large number of potential CNS phenomena are open to mischievous manipulations of peripheral (i.e. hormonal) endorphin fluctuations. 2. If they do not, as the body of available evidence indicates [the most provocative support *for* entry depends on unstated fractional entry values for wholly synthetic endorphin peptides not yet known to be functional agonists (148)], then blood-borne peptides must act at peripheral receptors. As indicated above, this could occur either through direct peripheral actions (see also 151) or through actions on parts of the CNS that are not guarded by the blood-brain barrier (152), including interactions with the hypothalamic projections to posterior pituitary (153, 154).

The pharmacological importance of the several members of the endorphin superfamily of peptides has thus scarcely been tapped. Current evidence suggests they may be involved, directly or indirectly, in a wide variety of important behavioral and vegetative physiological actions that extend well beyond the pharmacological actions of opiate drugs. Development of drugs that can exploit their broader potential physiological or pathophysiological involvement will require efforts at the molecular and cellular levels of analysis before drug effects on the whole animal can be meaningfully deciphered. Given the present momentum of the action, only a minimum amount of patience would seem necessary. The pharmacological analysis of the superfamily endorphin is likely to continue to serve as a model for the enlarging galaxy of new neuropeptide transmitters.

Literature Cited

1. Goldstein, A. 1976. Opioid peptides (endorphins) in pituitary and brain. *Science* 193:1081–86
2. Simon, E. J., Hiller, J. M. 1978. The Opiate Receptors. *Ann. Rev. Pharmacol. Toxicol.* 18:371–94
3. Terenius, L. 1978. Endogenous ptides and analgesia. *Ann. Rev. Pharmacol. Toxicol.* 18:189–204
4. Beaumont, A., Hughes, J. 1979. Biology of opioid ptides. *Ann. Rev. Pharmacol. Toxicol.* 19:245–67
5. Snyder, S. H., Childers, S. R. 1979. Opiate receptors and opioid ptides. *Ann. Rev. Neurosci.* 2:35–64
6. Watkins, L. R., Mayer, D. J. 1982. Organization of endogenous opiate and nonopiate pain control systems. *Science* 216:1185–92
7. Bolles, R. C., Fanselow, M. S. 1982. Endorphins and behavior. *Ann. Rev. Psychol.* 33:87–101
8. Berger, P. A., Akil, H., Watson, S. J., Barchas, J. D. 1982. Behavioral pharmacology of the endorphins. *Ann. Rev. Med.* 33:397–415
9. Emrich, H. M. 1981. *The Role of Endorphins in Neuropsychiatry.* Basel: Karger. 290 pp.
10. van Ree, J. M., De Wied, D. 1981. Endorphins in schizophrenia. *Neuropharmacology* 20:1271–77
11. Martinez, J. L. Jr., Jensen, R. A., Messing, R. B.,. Rigter, H., McGaugh, J. L. 1981. *Endogenous Peptides and Learning and Memory Processes.* New York: Academic
12. Koob, G. F., Bloom, F. E. 1982. Behavioral effects of neuropeptides: Endorphins and vasopressin. *Ann. Rev. Physiology* 44:571–82
13. Sawynok, J., P nsky, C., LaBella, F. S. 1979. Minireview on the specificity of naloxone as an opiate antagonist. *Life Sci.* 25:1621–32
14. Schwartz, J. C., Malfroy, B., Baume, S. D. L. 1981. Minireview: Biological inactivation of enkephalins and the role of enkephalin-dipeptidyl-carboxypeptidase ("Enkephalinase") as neuro ptidase. *Life Sci.* 29:1715–40
15. Hersh, L. B., Smith, T. E., McKelvy, J. F. 1980. Cleavage of endorphins to des-Tyr endorphins by homogeneous bovine brain amino ptidase. *Nature* 286: 160–62
16. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., Cohen, S. N., Numa, S. 1979. Nucleotide sequenced of cloned cDNA for bovine corticotropin β-lipotropin precursor. *Nature* 278:423–28
17. Roberts, J. L., Herbert, E. 1977. Characterization of a common precursor to corticotropin and β-lipotropin: cell-free synthesis of the precursor and identification of corticotropin peptides in the molecule. *Proc. Natl. Acad. Sci. USA* 74:4826–30
18. Roberts, J. L., Seeburg, P. H., Shine, J., Herbert, E., Baxter, J. D., Goodman, H. M. 1979. Corticotropin and β-endorphin: construction an analysis of recombinant DNA complementary to mRNA for the common precursor. *Proc. Natl. Acad. Sci. USA* 76:2153–57
19. Boileau, G., Seidah, N. G., Chretien, M. 1981. Biosynthesis of β-endorphin from proopiamelan ortin. In *Hormonal Proteins and Peptides,* ed. C. H. Li, 10:65–87. New York: Academic. 359 pp.
20. Shibasaki, T., Ling, N., Guillemin, R. 1980. Pituitary immunoreactive γ-melanotropins are glycosylated oligopeptides. *Nature* 285:416–17
21. Shibasaki, T., Ling, N., Guillemin, R., Silver, M., Bloom, F. E. 1981. The regional distribution of γ3-melanotropin-like ptides in bovine brain is correlated with adren orticotropin immunoreactivity but not with β-endorphin. *Regul. Peptides* 2:43–52
22. Bloom, F. E., Battenberg, E. L. F., Shibasaki, T., Benoit, R., Ling, N., Guillemin, R. 1980. Localization of γ-melanocyte stimulating hormone (γ MSH) immunoreactivity in rat brain and pituitary. *Regul. Peptides* 1:205–22
23. Zakarian, S., Smyth, D. G. 1982. Distribution of β-endorphin-related peptides in rat pituitary and brain. *Biochem. J.* 202:561–71
24. Rossier, J., Bloom, F. 1982. Central neuropharmacology of endorphins. In *Endorphins,* ed. J. B. Malick, R. M. S. Bell, pp. 165–86. New York: Dekker. 296 pp.
25. Guillemin, R., Ling, N., Lazarus, L., Burgus, R., Minick, S., Bloom, F., et al. 1977. The endorphins, novel ptides of brain and hypophysial origin, with opiate-like activity: biochemical and biologic studies. *Ann. N. Y. Acad. Sci.* 297:131–57
26. Verhoef, J., Wiegant, V. M., De Wied, D. 1982. Regional distribution of α- and γ-type endorphins in rat brain. *Brain Res.* 231:454–60
27. Pedigo, N. W., Ling, N. C., Reisine, T. D., Yamamura, H. I. 1979. Examina-

tion of des-tyrosine^1-γ-endorphin activity at ^{3}H-spiroperidol binding sites in rat brain. *Life Sci.* 24:1645–50
28. Versteeg, D. H. G., Kovacs, G. L., Bohus, B., De Kloet, E. R., De Wied, D. 1982. Effect of des-tyr^1-γ-endorphin and des-tyr^1-α-endorphin on α-MPT-induced catecholamine disappearance in rat brain nuclei: a dose-response study. *Brain Res.* 231:343–51
29. Lahti, R. A., Gay, D. D. 1982. Lack of effect of des-tyrosine^1-γ-endorphin on in vivo ^3_Hspiperone binding. *Eur. J. Pharmacol.* 80:127–29
30. Weinberger, S. B., Arnstein, A., Segal, D. C. 1979. Des-tyrosine-γ-endorphin and haloperidol: behavioral and biochemical differentiation. *Life Sci.* 24:1637–44
31. Weber, E., Evans, C. J., Barchas, J. D. 1981. Acetylated and nonacetylated forms of β-endorphin in rat brain and pituitary. *Biochem. Biophysical Res. Commun.* 103:982–89
32. Gubler, U., Seeburg, P., Hoffman, B. J., Gage, L. P., Udenfriend, S. 1982. Molecular cloning establishes proenkephalin as precursor of enkephalin-containing peptides. *Nature* 295:206–8
33. Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Hirose, T., Inayama, S., Nakanishi, S., Numa, S. 1982. Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. *Nature* 295:202–6
34. Comb, M., Seeburg, P. H., Adelman, J., Eiden, L., Herbert, E. 1982. Primary structure of the human Met- and Leu-enkephalin precursor and its mRNA. *Nature* 295:663
35. Yang, H.-Y. T., Hexum, T., Costa, E. 1980. Minireview opioid peptides in adrenal gland. *Life Sci.* 27:1119–25
36. Hokfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J., Schultzberg, M. 1980. Peptidergic neurones. *Nature* 284: 515–21
37. Bloom, F. E., McGinty, J. F. 1981. Cellular distribution and function of endorphins. In *Endogenous Peptides and Learning and Memory processes*, pp. 199–230. New York: Academic. 587 pp.
38. Goldstein, A., Tachibana, S., Lowney, L. I., Hunkapiller, M., Hold, L. 1979. Dynorphin-(1-13,) an extraordinarily potent opioid peptide. *Proc. Natl. Acad. Sci. USA* 76:6666–70
39. Goldstein, A., Fischli, W., Lowney, L. I., Hunkapiller, M., Hood, L. 1981. Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. *Proc. Natl. Acad. Sci. USA* 78:7219–23
40. Tachibana, S., Araki, K., Ohya, S., Yoshida, S. 1982. Isolation and structure of dynorphin, an opioid peptide, from porcine duodenum. *Nature* 295: 339–40
41. Watson, S. J., Akil, H., Ghazarossian, V. E., Goldstein, A. 1981. Dynorphin immunocytochemical localization in brain and peripheral nervous system: preliminary studies. *Proc. Natl. Acad. Sci. USA* 78:1260–63
42. Chavkin, C., James, I. F., Goldstein, A. 1982. Dynorphin is a specific endogenous ligand of the k opioid receptor. *Science* 215:413–15
43. Oka, T., Negishi, K., Kajiwara, M., Watanabe, Y., Ishizuka, Y., Matsumiya, T. 1982. The choice of opiate receptor subtype by neoendorphins. *Eur. J. Pharmacol.* 79:301–5
44. Kangawa, K., Minamino, N., Chino, N., Sakakibara, S., Matsuo, H. 1981. The complete amino acid sequence of α-neoendorphin *Biochem. Biophys. Res. Commun.* 99:871–78
45. Weber, E., Roth, K. A., Barchas, J. D. 1981. Colocalization of α-neoendorphin and dynorphin immunoreactivity in hypothalamic neurons. *Biochem. Biophys. Res. Commun.* 103:951–58
46. Weber, E., Roth, K. A., Barchas, J. D. 1982. Immunohistochemical distribution of α-neoendorphin/dynorphin neuronal systems in rat brain: evidence for colocalization. *Proc. Natl. Acad. Sci. USA* 79:3062–66
47. Minamino, N., Kangawa, K., Chino, N., Sakakibara, S., Matsuo, H. 1981. β-neoendorphin, a new hypothalamic "big" leu-enkephalin of porcine origin: its purification and the complete amino acid sequence. *Biochem. Biophys. Res. Commun.* 99:864–70
48. Sar, M., Stumpf, W. E., Miller, R. J., Chang, K.-J., Cuatrecasas, P. 1978. Immunohistochemical localization of enkephalin in rat brain and spinal cord. *J. Comp. Neurol.* 182:17–38
49. McGinty, J. F., Henriksen, S. J., Goldstein, A., Terenius, L., Bloom, F. E. 1982. Opioid peptide identity and localization in hippocampus. *Life Sci.* In press
50. Gall, C., Brecha, N., Karten, H. J., Chang, K.-J. 1981. Localization of enkephalin-like immunoreactivity to identified axonal and neuronal populations of the rat hippocampus. *J. Comp. Neurol.* 198:335–50

51. Wuster, M., Rubini, P., Schulz, R. 1981. The preference of putative proenkephalins for different types of opiate receptors. *Life Sci.* 29:1219–27
52. Rossier, J., Audigier, Y., Ling, N., Cros, J., Udenfriend, S. 1980. Met-enkephalin-Arg^6-Phe^7, present in high amounts in brain of rat, cattle, and man, is an opioid agonist. *Nature* 288:88–89
53. Greenberg, M. J., Price, D. A. 1980. Cardioregulatory peptides in molluscs. In *Peptides: Integrators of Cell and Tissue Function,* ed. F. E. Bloom, pp. 107–26. New York: Raven. 257 pp.
54. Weber, E., Evans, C. J., Samuelsson, S. J., Barchas, J. D. 1981. Novel peptide neuronal system in rat brain and pituitary. *Science* 214:1248–51
55. Dockray, G. J., Vaillant, C., Williams, R. G. 1981. New vertebrate brain-gut peptide related to a molluscan neuropeptide and an opioid peptide. *Nature* 293:656–57
56. Cottrell, G. A. 1982. FMRFamide neuropeptides simultaneously increase and decrease K^+ currents in an identified neurone. *Nature* 296:87–89
57. Alumets, J., Hakanson, R., Sundler, F., Thorell, J. 1979. Neuronal localization of immunoreactive enkephalin and α-endorphin in the earthworm. *Nature* 279:805–6
58. Zipser, B. 1980. Identification of specific leech neurones immunoreactive to enkephalin. *Nature* 283:857–58
59. Mancillas, J. R., McGinty, J. F., Selverston, A. I., Karten, H., Bloom, F. E. 1981. Immunocytochemical localization of enkephalin and substance P in retina and eyestalk neurones of lobster. *Nature* 293:576–78
60. Gozzini, L., Montecucchi, P. C. 1981. Reversed-phase high-performance liquid chromatography of dermorphins, opiate-like peptides from amphibian skins. *J. Chromatogr.* 216:355–60
61. Henschen, A., Lottspeich, F., Brantl, V., Teschemacher, H. 1979. Novel opioid peptides derived from casein (β-casomorphins.) *Hoppe-Seylers Z. Physiol. Chem.* 360:1217–24
62. Takagi, H., Shiomi, H., Ueda, H., Amano, H. 1979. A novel analgesic dipeptide from bovine brain is a possible Met-enkephalin release. *Nature* 282: 410–12
63. Sarne, Y., Azov, R., Weissman, B. A. 1978. A stable enkephalin-like immunoreactive substance in human CSF. *Brain Res.* 151:399–403
64. LeRoith, D., Liotta, A. S., Roth, J., Shiloach, J., Lewis, M. E., Pert, C. B., Krieger, D. T. 1981. Corticotropin and β-endorphin-like materials are native to unicellular organisms. *Proc. Natl. Acad. Sci. USA* 79:2086–90
65. Simantov, R., Kuhar, M. J., Uhl, G. R., Snyder, S. H. 1977. Opioid peptide enkephalin: immunohistochemical mapping in rat central nervous system. *Proc. Natl. Acad. Sci. USA* 74:2167–71
66. Kobayashi, R. M., Palkovits, M., Miller, R. J., Chang, K.-J., Cuatrecasas, P. 1978. Brain enkephalin distribution is unaltered by hypophysectomy. *Life Sci.* 22:527–30
67. Gramsch, C., Hollt, V., Pasi, A., Mehraein, P., Herz, A. 1982. Immunoreactive dynorphin in human brain and pituitary. *Brain Res.* 233:65–74
68. Hong, J. S., Yang, H. Y., Fratta, W., Costa, E. 1977. Determination of methionine enkephalin in discrete regions of rat brain. *Brain Res.* 134:383–86
69. Dupont, A., Lepine, J., Langelier, P., Merand, Y., Rouleau, D., Vaudry, H., Gros, C., Barden, N. 1980. Differential distribution of β-endorphin and enkephalins in rat and bovine brain. *Regul. Peptides* 1:43–52
70. Uhl, G. R., Kuhar, M. J., Snyder, S. H. 1978. Enkephalin-containing pathway: amygdaloid efferents in the stria terminalis. *Brain Res.* 149:223–28
71. Wamsley, J. K., Young, W. S. III, Kuhar, M. J. 1980. Immunohistochemical localization of enkephalin in rat forebrain. *Brain Res.* 190:153–74
72. Bayon, A., Koda, L., Battenberg, E., Bloom, F. 1980. Redistribution of endorphin and enkephalin immunoreactivity in the rat brain and pituitary after in vivo treatment with colchicine or cytochalasin 3. *Brain Res.* 183: 103–11
73. Bayon, A., Shoemaker, W. J., McGinty, J., Bloom, F. E. 1982. Immunodetection of endorphins and enkephalins: a search for reliability. In *International Review of Neurobiology.* New York: Academic. In press
74. Bayon, A., Koda, L., Battenberg, E., Azad, R., Bloom, F. E. 1980. Regional distribution of endorphin, met-enkephalin and leu-enkephalin in the pigeon brain. *Neurosci. Lett.* 16:75–80
75. Pickel, V. M., Sumal, K. K., Beckley, S. C., Miller, R. J., Reis, D. J. 1980. Immunocytochemical localization of enkephalin in the neostriatum of rat brain: a light and electron microscopic study. *J. Comp. Neurology* 189:721–40
76. Hunt, S. P., Kelly, J. S., Emson, P. C. 1980. The electron microscopic locali-

zation of methionine-enkephalin within the superficial layers (I and II) of the spinal cord. *Neuroscience* 5:1871–90
77. Aronin, N., DiFiglia, M., Liotta, A. S., Martin, J. B. 1981. Ultrastructural localization and biochemical features of immunoreactive leu-enkephalin in monkey dorsal horn. *J. Neurosci.* 1: 561–77
78. DiFiglia, M., Aronin, N., Martin, J. B. 1982. Light and electron microscopic localization of immunoreactive leu-enkephalin in the monkey basal ganglia. *J. Neurosci.* 2:303–20
79. McGinty, J. F., van der Kooy, D., Koda, L. Y., Bloom, F. E. 1982. Enkephalin immunoreactive cells in frontal, olfactory, and limbic cortex. *Anat. Rec.* 202:125A
80. Schulman, J. A., Finger, T. E., Brecha, N. C., Karten, H. J. 1981. Enkephalin immunoreactivity in golgi cells and mossy fibres of mammalian, avian, amphibian and teleost cerebellum. *Neuroscience.* 6:2407–16
81. Hokfelt, T., Terenius, L., Kuypers, H. G. J. M., Dann, O. 1979. Evidence for enkephalin immunoreactive neurons in the medulla oblongata projecting to the spinal cord. *Neurosci. Lett.* 14:55–60
82. Goldstein, A., Ghazarossian, V. E. 1980. Immunoreactive [redacted] in pituitary and brain. *Proc[redacted]ad. Sci. USA* 77:6207–10
83. Cuello, A. C., Paxinos, G. 1978. Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature* 271:178–80
84. Correa, F. M. A., Innis, R. B., Hester, L. D., Snyder, S. H. 1981. Diffuse enkephalin innervation from caudate to globus pallidus. *Neurosci. Lett.* 25: 63–68
85. Rossier, J., Battenberg, E., Pittman, Q., Bayon, A., Koda, L., Miller, R., Guillemin, R., Bloom, F. 1979. Hypothalamic enkephalin neurones may regulate the neurohypophyses. *Nature* 277:653–55
86. Girod, C., Dubois, M. P., Durand, N. 1981. Somatostatin-immunoreactive and met-enkephalin-immunoreactive fibers in neurohypophysis of monkeys macaca irus and papio hamadryas. *Neuroendocrinol. Lett.* 3:153–58
87. Micevych, P., Elde, R. 1980. Relationship between enkephalinergic neurons and the vasopressin-oxytocin neuroendocrine system of the cat: an immunohistochemical study. *J. Comp. Neurol.* 190:135–46
88. Watson, S. J., Akil, H., Fischli, W., Goldstein, A., Zimmerman, E., Nilaver, G., van Wimersma Greidanus, T. B. 1982. Dynorphin and vasopressin: common localization in magnocellular neurons. *Science* 216:85–87
89. Baetge, G., Shoemaker, W. J., Bayon, A., Azad, R., Bloom, F. E. 1982. Transient visualization of β-endorphin-containing neurons in the rat brain stem during perinatal development. *Soc. Neurosci.* In press
90. Chang, K.-J., Cuatrecasas, P. 1981. Heterogeneity and properties of opiate receptors. *Fed. Proc.* 40:2729–34
91. Martin, W. R. 1981. Mini-symposium. II. Multiple opioid receptors: a little about the history and some implications related to evolution. *Life Sci.* 28: 1547–54
92. Zukin, R. S., Zukin, S. R. 1981. Minireview: multiple opiate receptors: emerging concepts. *Life Sci.* 29:2681–90
93. North, R. A. 1979. Minireview, opiates, opioid peptides and single neurones. *Life Sci.* 24:1527–46
94. Williams, J. T., Zieglgansberger, W. 1981. Neurons in the frontal cortex of the rat carry multiple opiate receptors. *Brain Res.* 226:304–8
95. Siggins, G. R., Zieglgansberger, W. 1981. Morphine and opioid peptides reduce inhibitory synaptic potentials in hippocampal pyramidal cells in vitro without alteration of membrane potential. *Proc. Natl. Acad. Sci. USA* 78: 5235–39
96. Nicoll, R. A., Alger, B. E., Jahr, C. E. 1980. Enkephalin blocks inhibitory pathways in the vertebrate CNS. *Nature* 287:22–25
97. Goodman, R. R., Snyder, S. H., Kuhar, M. J., Young, W. S. III. 1980. Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. *Proc. Natl. Acad. Sci. USA* 77:6239–43
98. Herkenham, M., Pert, C. B. 1980. In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways. *Proc. Natl. Acad. Sci. USA* 77:5532–36
99. Lewis, M. E., Mishkin, M., Bragin, E., Brown, R. M., Pert, C. B., Pert, A. 1981. Opiate receptor gradients in monkey cerebral cortex: correspondence with sensory processing hierarchies. *Science* 211:1166–69
100. Young, W. S., Kuhar, M. J. 1979. A new method for receptor autoradiography: ^{3}H-opioid receptors in rat brain. *Brain Res.* 179:255–70
101. Palmer, M. R., Hoffer, B. J. 1980. Catecholamine modulation of enkephalin-

induced electrophysiological responses in cerebral cortex. *J. Pharm. Exper. Ther.* 213:205–15
102. Nakamura, S., Tepper, J. M., Young, S. J., Ling, N., Groves, P. M. 1982. Noradrenergic terminal excitability: effects of opioids. *Neurosci. Lett.* 30:57–62
103. Egan, T. M., North, R. A. 1981. Both μ and α opiate receptors exist on the same neuron. *Science* 214:923–24
104. Szalay, K. S., Stark, E. 1981. Effect of beta-endorphin on the steroid production of isolated zona glomerulosa and zona fasciculata cells. *Life Sci.* 29: 1355–61
105. Haddox, M. K., Russell, D. H. 1979. β-endorphin is a kidney trophic hormone. *Life Sci.* 25:615–20
106. Hazum, E., Chang, K.-J., Cuatrecasas, P. 1979. Specific nonopiate receptors for β-endorphin. *Science* 205:1033–35
107. Schweigerer, L., Bhakdi, S., Teschemacher, H. 1982. Specific non-opiate binding sites for human β-endorphin on the terminal complex of human complement. *Nature* 296:572–74
108. Gilman, S. C., Schwartz, J. M., Milner, R. J., Bloom, F. E., Feldman, J. D. 1982. Â-endorphin enhances lymphocyte proliferative responses. *Proc. Natl. Acad. Sci. USA* 79:4226–30
109. Nicoll, R. A. 1982. Responses of central neurons to opiates and opioid peptides. In *Pharmacological and Physiologic Considerations,* ed. M. Trabucchi, E. Costa. New York: Raven. In press
110. Siggins, G. R., McGinty, J. F., Morrison, J. H., Pittman, Q. J., Zieglgansberger, W., Magistretti, P. J., Gruol, D. L. 1982. The role of neuropeptides in the hippocampal formation. In *Adv. Biochem. Psychopharmacol.,* ed. M. Trabucchi, E. Costa. New York: Raven. In press
111. Wouters, W., van den Bercken, J. 1979. Hyperpolarisation and depression of slow synaptic inhibition by enkephalin in frog sympathetic ganglion. *Nature* 277:53–54
112. Konishi, S., Tsunoo, A., Otsuka, M. 1981. Enkephalin as a transmitter for presynaptic inhibition in sympathetic ganglia. *Nature* 294:80–82
113. Pepper, C. M., Henderson, G. 1980. Opiates and opioid peptides hyperpolarize locus coeruleus neurons in vitro. *Scien e* 209:394–96
114. Barker, J. L., Smith, T. G. 1979. Three modes of communication in the nervous system. *Adv. Exp. Med. Biol.* 116:3–25
115. Zieglgansberger, W., Tulloch, I. F. 1979. The effects of methionine- and leucine-enkephalin on spinal neurones of the cat. *Brain Res.* 167:53–64
116. Davies, J., Dray, A. 1978. Pharmacological and electrophysiological studies of morphine and enkephalin on rat supraspinal neurones and cat spinal neurones. *Br. J. Pharmacol.* 63:87–96
117. Satoh, M., Akaike, A., Takagi, H. 1979. Excitation by morphine and enkephalin of single neurones of nucleus reticularis paragigantocellularis in the rat: a probable mechanism of analgesic action of opioids. *Brain Res.* 169: 406–10
118. Henriksen, S. J., Chouvet, G., McGinty, J., Bloom, F. E. 1982. Neuropeptides and the epilepsies: current perspectives. In *World Health Organization Epilepsy Meeting Book.* In press
119. Henriksen, S. J., Bloom, F. E., McCoy, F., Ling, N., Guillemin, R. 1978. β-endorphin induces nonconvulsive limbic seizures. *Proc. Natl. Acad. Sci. USA* 75:5221–25
120. Zieglgansberger, W., French, E., Siggins, G. R., Bloom, F. E. 1979. Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. *Science* 205: 415–17
121. Cotman, C. 1981. *Regulatory Mechanism of Synaptic Transmission.* New York: Plenum. 422 pp.
122. Schulz, R., Wuster, M., Herz, A. 1979. Supersensitivity of opioids following the chronic blockade of endorphin action by naloxone. *Naunyn-Schmiedebergs Arch. Pharmak.* 306:93–96
123. Johnson, S. M., Duggan, A. W. 1981. Tolerance and dependence of dorsal horn neurones of the cat: the role of the opiate receptors of the substantia gelatinosa. *Neuropharmocology* 20: 1033–38
124. Zieglgansberger, W., Fry, J. P., Herz, A., Moroder, L., Wunsch, E. 1976. Enkephalin-induced inhibition of cortical neurones and the lack of this effect in morphine tolerant/dependent rats. *Brain Res.* 115:160–164
125. Chang, K.-J., Cuatrecasas, P., Wei, E. T., Chang, J.-K. 1982. Analgesic activity of intracerebroventricular administration of morphiceptin and β-casomorphins: correlation with the morphine (μ) receptor binding affinity. *Life Sci.* 30:1547–51
126. Gillin, J. C., Hong, J. S., Yang, H.-Y. T., Costa, E. 1978. [Met^5] enkephalin content in brain regions of rats treated with lithium. *Proc. Natl. Acad. Sci. USA* 75:2991–93

127. Hong, J. S., Yang, H.-Y. T., Gillin, J. C., DiGiulio, A. M., Fratta, W., Costa, E. 1979. Chronic treatment with haloperidol accelerates the biosynthesis of enkephalins in rat striatum. *Brain Res.* 160:192–95
128. Fratta, W., Rossetti, Z., Gessa, G. L. 1981. Rapid depletion and restoration of striatal met-enkephalin after ketamine. *Life Sci.* 29:93–97
129. Hollt, V., Bergmann, M. 1982. Effects of acute and chronic haloperidol treatment on the concentrations of immunoreactive β-endorphin in plasma, pituitary and brain of rats. *Neuropharmacology* 21:147–54
130. Harsing, L. G. Jr., Yang, H.-Y. T., Govoni, S., Costa, E. 1982. Elevation of met^5-enkephalin and β-endorphin hypothalamic content in rats receiving anorectic drugs: differences between d-fenfluramine and d-amphetamine. *Neuropharmacology* 21:141–45
131. Staunton, D. A., Deyo, S. N., Shoemaker, W. J., Ettenberg, A., Bloom, F. E. 1982. Effects of chronic dietary lithium exposure on rat brain enkephalin systems. *Soc. Neurosci. Abstr.* 8. In press
132. Pfeiffer, A., Seizinger, B. R., Herz, A. 1981. Chronic ethanol inhibition interferes with δ-, but not with μ-opiate receptors. *Neuropharmacology* 20: 1229–32
133. Moroni, F., Cheney, D. L., Costa, E. 1977. β-endorphin inhibits ACh turnover in nuclei of rat brain. *Nature* 267:267–68
134. Botticelli, L. J., Wurtman, R. J. 1979. β-endorphin administration increases hippocampal acetylcholine levels. *Life Sci.* 24:1799–804
135. Hui, K.-S., Graf, L., Lajtha, A. 1982. β-endor hin inhibits met-enkephalin breakdown by a brain aminopeptidase: structure-activity relationships. *Biochem. Biophys. Res. Commun.* 105: 1482–87
136. Russell, J., Bass, P., Goldberg, L. I., Schuster, C. R., Merz, H. 1982. Antagonism of gut, but not central effects of morphine with quaternary narcotic antagonists. *Eur. J. Pharm.* 78: 255–61
137. Ferretti, P., Bianchi, G., Tavani, A., Manara, L. 1981. Inhibition of gastrointestinal transit and antinociceptive effects of morphine and FK 33–824 in rats are differently prevented by naloxone and by its N-methyl quaternary analog. *Res. Commun. Subst. Abuse* 2:1
138. Holaday, J. W., Faden, A. I. 1980. Naloxone acts at central opiate receptors to reverse hypotension, hypothermia and hypoventilation in spinal shock. *Brain Res.* 189:295–99
139. The Editor. 1981. Naloxone for septic shock. *The Lancet*: 538–39
140. Hosobuchi, Y., Baskin, D. S. 1982. Reversal of induced ischemic neurologic deficit in gerbils by the opiate antagonist naloxone. *Science* 215:69–71
141. Clark, W. G. 1981. Effects of opioid peptides on thermoregulation. *Fed. Proc.* 40:2754–59
142. Ettenberg, A., Rogers, J., Koob, G. F., Bloom, F. E., Deutsch, J. A. 1981. Endogenous opiates and fasting. *Science* 213:1282
143. Gambert, S. R., Garthwaite, T. L., Pontzer, C. H., Hagen, T. C. 1981. Endogenous opiates and fasting. *Science* 213:1282
144. Brown, D. R., Holtzman, S. G. 1981. Opiate antagonists: central sites of actin in suppressing water intake of the rat. *Brain Res.* 221:434–36
145. Jeffcoate, W. J., Herbert, M., Cullen, M. H., Hastings, A. G., Walder, C. P. 1979. Prevention of effects of alcohol intoxication by naloxone. *Lancet* 2: 1157–59
146. Carr, D. B., Bullen, B. A., Skrinar, G. S., Arnold, M. A., Rosenblatt, M., et al. 1981. Physical conditioning facilitates the exercise-induced secretion of beta-endorphin and beta-liptropin in women. *N. Engl. J. Med.* 305:560–63
147. Tseng, L.-F., Loh, H. H., Li, C. H. 1976. β-endorphin as a potent analgesic by intravenous injection. *Nature* 263: 239–40
148. Rapoport, S. I., Klee, W. A., Pettigrew, K. D., Ohno, K. 1979. Entry of opioid peptides into the central nervous system. *Science* 207:84–86
149. Foley, K. M., Kourides, I. A., Inturrisi, C. E., Kaiko, R. F., Zaroulis, C. G., et al. 1979. β-endorphin: analgesic and hormonal effects in humans. *Proc. Natl. Acad. Sci. USA* 76:5377–81
150. Houghten, R. A., Swann, R. W., Li, C. H. 1980. β-endorphin: stability, clearance behavior, and entry into the central nervous system after intravenous injection of the tritiated peptide in rats and rabbits. *Proc. Natl. Acad. Sci. USA* 177:4588–91
151. Knepel, W., Nutto, D., Anhut, H., Hertting, G. 1982. Vasopressin and β-endorphin release after osmotic and non-osmotic stimuli: effect of naloxone

and dexamethasone. *Eur. J. Pharmacol.* 77:299–306
152. Schaz, K., Stock, G., Simon, W., Schlor, K-H., Unger, T., et al. 1980. *Hypertension* 2:395
153. Iversen, L. L., Iversen, S. D., Bloom, F. E. 1980. Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis. *Nature* 284:350–51
154. Lightman, S. L., Iversen, L. L., Forsling, M. L. 1982. Dopamine and [D-ALA^2,d-Leu^5] enkephalin inhibit the electrically stimulated neurohypophyseal release of vasopressin in vitro: eviden e for calcium-dependent opiate action. *J. Neurosci.* 2:78–81