Peptides, Vol. 1, Suppl. 1, pp. 11–20. Printed in the U.S.A.

Anatomical and Biochemical Studies of the Opioid Peptides and Related Substances in the Brain[1]

STANLEY J. WATSON, HUDA AKIL AND J. MICHAEL WALKER

Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

WATSON, S. J., H. AKIL AND J. M. WALKER. *Anatomical and biochemical studies of the opioid peptides and related substances in the brain.* PEPTIDES 1: Suppl. 1, 11–20, 1980.—It is now clear that there are at least four opioid peptide-neuronal systems in mammalian brain: the enkephalins, beta-endorphin and dynorphin. The focus of this presentation will be twofold—to focus on the multiple transmitter problem as typified by the beta-END/alpha-MSH arcuate neuronal system, and to describe the newly-discovered dynorphin neuronal system. The beta-END/alpha-MSH neurons have been studied using antisera directed against different portions of the 31K precursor (ACTH/beta-LPH/beta-END). Although ACTH can be detected in brain, the final products of the brain 31K system seem to be beta-END and alpha-MSH (similar to the intermediate lobe of pituitary). It is emphasized that "normally" these neurons would appear to release two neuronally active substances. Recently, a second set of alpha-MSH immunoreactive neurons was discovered in rat brain. These neurons are not immunoreactive for any other part of the 31K precursor and are distributed quite differently than the arcuate beta-END/alpha-MSH cell group. Dynorphin is another major class of opioid peptide. It has been studied and found in magnocellular neurons and posterior pituitary. The relations between dynorphin and leu-enkephalin cells continues to be unclear.

Opiate peptides β-Endorphin Dynorphin ACTH α-MSH Immunocytochemistry Anatomy

THE following chapter will present an overview of anatomical and physiological information regarding the several opiate peptides discovered to date—beta-endorphin, methionine enkephalin, leucine enkephalin and dynorphin. Each of these peptides appears to be contained in its own neuronal system. At the same time they seem to be related in one fashion or another to other neurotransmitter and specifically other peptide systems. The perspective of this chapter will be to highlight the characteristics of these opioid peptide systems with particular attention being paid to problems, tools, and novel or unusual perspectives. The primary information from which most of these observations are drawn are the basic immunocytochemical and anatomical studies carried out by several laboratories including our own. Additionally, other classes of information will be brought into the discussion as they are relevant to understanding the complexities of the system. For example, in the beta endorphin system we will present some biochemical, pharmacological and behavioral studies. This convergence of studies has proven to be a powerful tool for elucidating the function of this system in brain.

The Beta-Endorphin/ACTH/Alpha-MSH/Gamma-MSH System

The first study on the system involving beta-lipotropin was carried out by Moon and co-workers in the early '70's [29]. In that study it was shown that beta-lipotropin was distributed in pituitary in a fashion very similar to ACTH and alpha-MSH. That is, beta-lipotropin was stored in all of the cells of the intermediate lobe as well as the corticotrophs of the anterior lobe. With the discovery of beta-endorphin [9, 17, 26] and the development of antisera against it, it was possible to confirm that ACTH, beta-endorphin and beta-lipotropin were stored in precisely the same locations in pituitary [7,36]. Soon thereafter immunocytochemical studies of lipotropin and endorphin were extended to brain with the discovery of a separate and unique neuronal pool in central nervous system which contained both beta endorphin and beta lipotropin [6, 8, 52, 54, 57]. These neurons are located in the region of the arcuate nucleus of hypothalamus and contain fiber systems which project widely throughout the brain stem. The association of lipotropin and endorphin with ACTH and α-MSH in two pituitary cell groups strongly suggested the likelihood that there might be ACTH immunoreactivity in brain also related to lipotropin and endorphin.

Subsequently we [55] and others [22] carried out studies of ACTH immunoreactivity in central nervous system. We were able to locate it in precisely the same cells which contain beta-endorphin and beta-lipotropin [52]. These im-

[1]This work was supported in part by NIDA grant number DA 02265, NSF grant number BNS 8004512 (to HA and SW) and NIDA postdoctoral grant IF 32DA05183 (to MW).

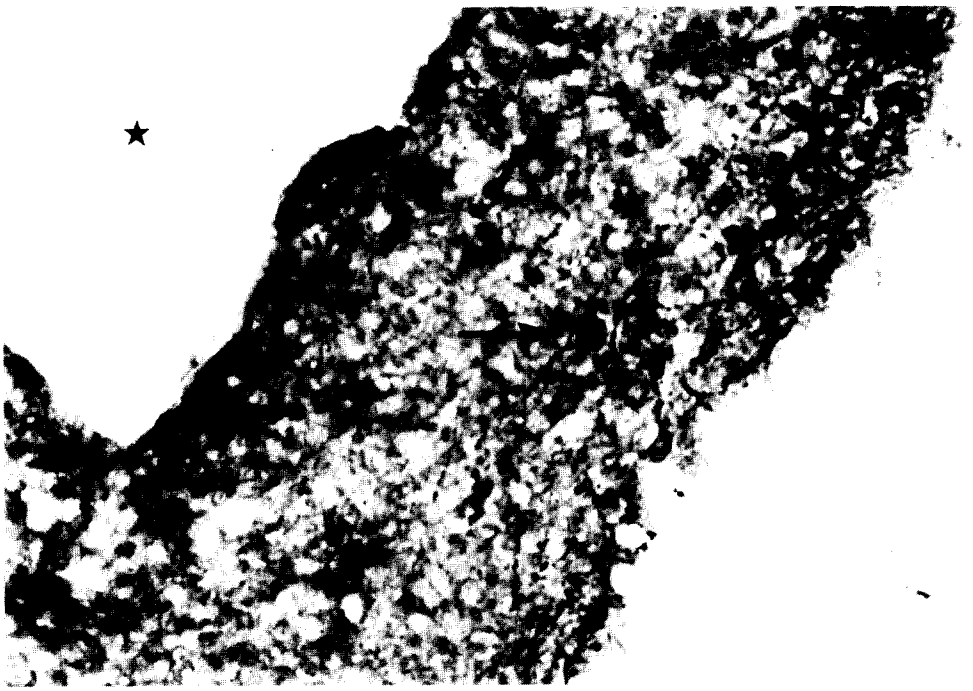

FIG. 1. β-END cells (arrow) in the extreme posterior arcuate nucleus. Star is in the third ventricle. ×300.

munocytochemical studies were carried out after the elegant studies of Mains and Eipper [28], and Roberts and Herbert [39] in which they demonstrated the presence of a single major protein precursor molecule for ACTH, beta-endorphin and beta-lipotropin. Thus the theoretical linkage between endorphins, lipotropin and ACTH was strongly reinforced by the discovery of a common precursor molecule in pituitary. This molecule was shown to contain beta-lipotropin and beta-endorphin at the C terminus, ACTH in the mid portion of the molecule and a fragment at the N terminus of unknown structure or function (called the 16K portion) [28]. The 31,000 Dalton precursor molecule has since come to be known as either the 31K precursor, pro-opiocortin or pro-opiomelanocortin. This elegant protein biochemical work on the 31K precursor [28,39] has been confirmed and expanded by the work of Nakanishi and coworkers [30] using molecular biological tools. These workers determined the cDNA sequence from the pro-opiocortin messenger RNA suggesting a structure which, in every important aspect, is identical to the sequence obtained from biochemical studies. They further revealed the amino acid composition and sequence of the 16K structure, gamma-MSH and of the signal peptide.

Upon more careful evaluation of the distribution of the 31K precursor and its products in pituitary, it was determined that the two lobes of pituitary treated the 31K precursor in a different fashion [12,15]. The anterior lobe cells which produced ACTH were found to process pro-opiocortin into full ACTH with a modest amount of beta-endorphin and a larger amount of beta-lipotropin. In contrast, intermediate lobe cells carried the cleavage one step further. ACTH was cleaved into alpha-MSH (N-acetyl ACTH 1–13 amide) and CLIP (corticotropin-like intermediate lobe peptide); whereas beta-lipotropin was very rapidly and almost completely cleaved into gamma-lipotropin and beta-endorphin. Thus the pituitary appeared to use the same general precursor information to make two different sets of products. The question remained as to whether brain acted more like anterior lobe or intermediate lobe. In subsequent studies using specific ACTH antisera and a serial section analysis we and others determined that the same cells in brain did produce ACTH, beta-lipotropin and β-endorphin [4, 31, 52]. However, since most of the ACTH antibodies used in these studies could also bind to α-MSH or CLIP, the question of which peptide was being studied had to be investigated. Other groups had used specific α-MSH antisera and shown its presence in the CNS [32, 35, 46]. We extended these studies to show that all of the alpha-MSH positive cells in the arcuate nucleus produced beta endorphin as well [49,50]. Thus, it became clear that there was a very similar precursor-product system in brain and pituitary. That is, brain contains most of the protein pieces that one would expect for cleavage from the 31K precursor and that these pieces were stored within the same cells. With the development of antisera against the 16K portion of the 31K precursor and gamma-MSH it has been possible to reinforce this finding by demonstrating its existence in the beta-END containing neurons and pituitary cells [5,34].

As an interesting aside, while studying central nervous system distribution of alpha-MSH, a second set of cells with alpha-MSH immunoreactivity has been discovered [49,50]. This cell group is completely outside of arcuate nucleus but is still located in hypothalamus. It stretches from the top of the third ventricle in a dorsal lateral fashion out to the posterior regions of the hypothalamus ending with a subgroup abutting against the lateral hypothalamic sulcus. This set of alpha-MSH positive cells is not positive for beta-endorphin, the C terminal of ACTH, gamma-lipotropin or 16K, suggesting that the neurons contain an alpha-MSH-like peptide but do not seem to store other portions of the 31K molecule.

In looking back over the 31K beta-endorphin/ACTH/MSH system it becomes clear that there is an enor-

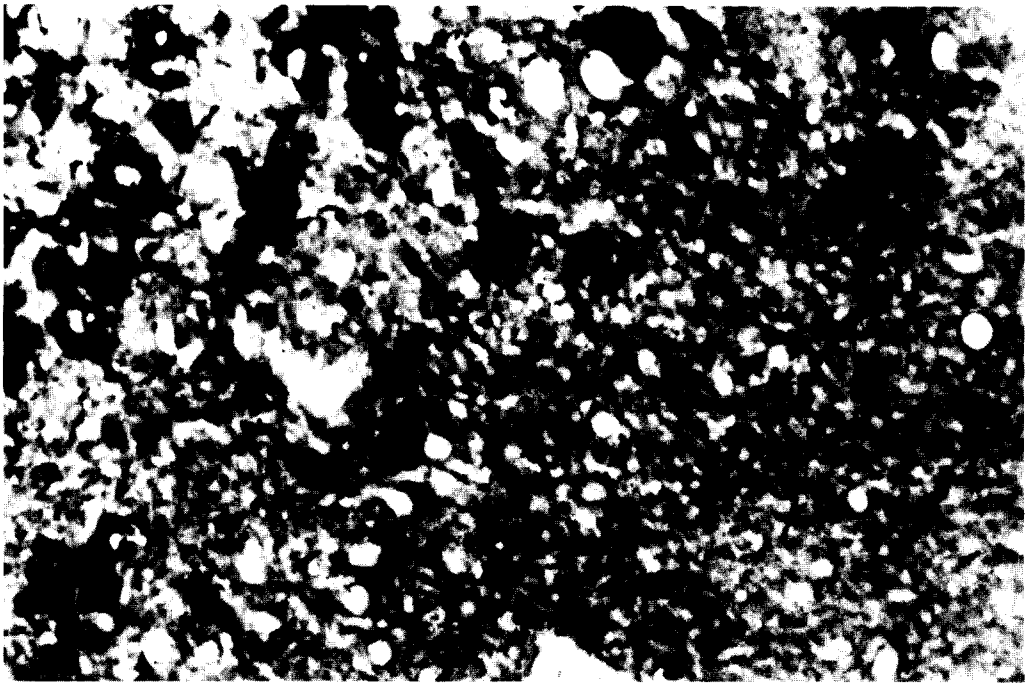

FIG. 2. α-MSH cells in the arcuate nucleus. ×540.

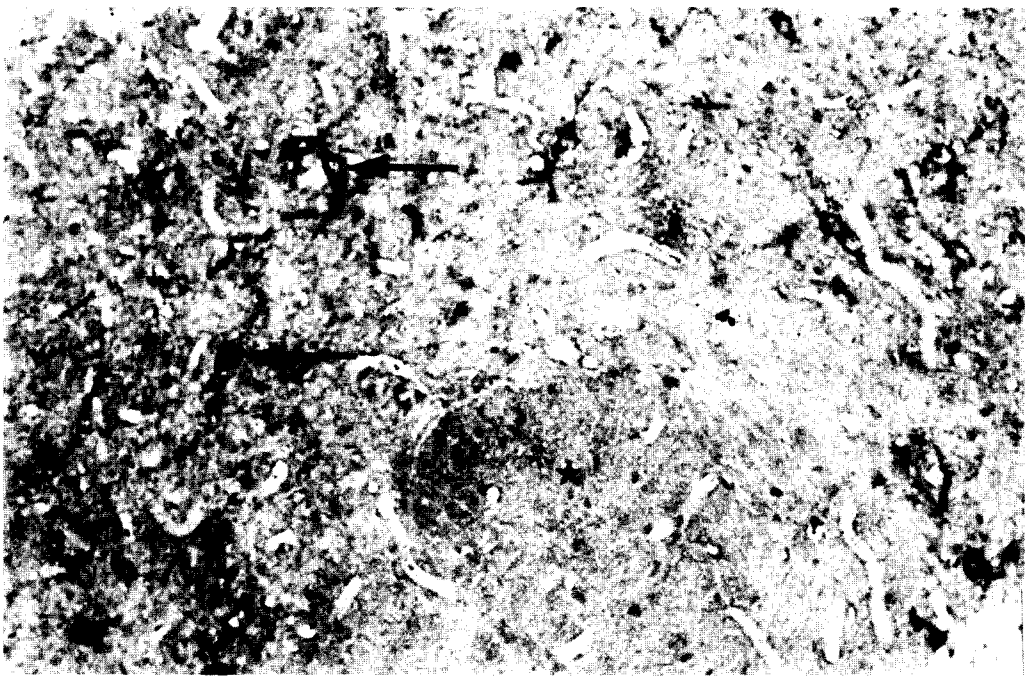

FIG. 3. α-MSH cells (arrows) near the fornix (star). These cells are not part of the arcuate β-END system. They only stain with α-MSH antisera. ×250.

mous complexity inherent in the biology of this precursor molecule. It is not only found in three different cell regions (anterior lobe and intermediate lobe of pituitary as well as arcuate nucleus of hypothalamus), but it produces several potentially active biological products within the same cell. Whether these substance would all qualify as active neurotransmitters requires studies of their release, receptors, physiological and behavioral effects. Only a few studies have begun to address such issues. We shall focus here on work from our laboratory, which is beginning to shed light on the function of the brain 31K system. Our studies in human pain patients have strongly suggested that endorphin and alpha-MSH can be released into the CSF by electrical stimulation of periventricular sites known to produce relief of intractable pain [2, 3, 37, 38]. Similar studies by Hosobuchi and collaborators [18] have shown the release of N terminal portions

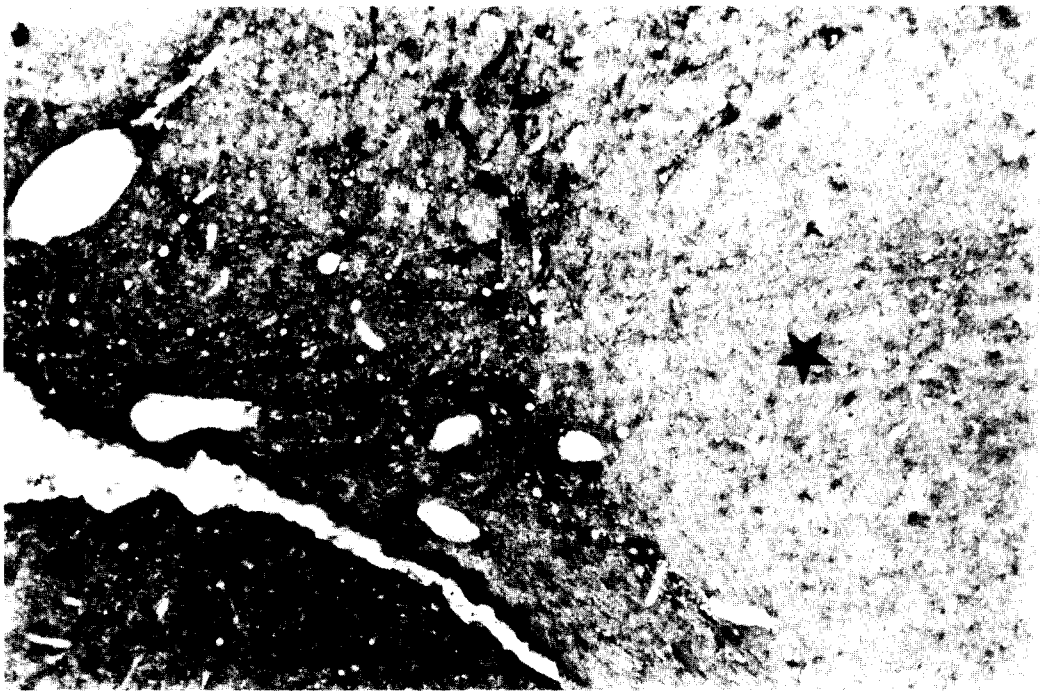

FIG. 4. α-MSH cells (arrows) in the lateral hypothalamus near the optic tract (star). These cells do not stain for β-END. ×175.

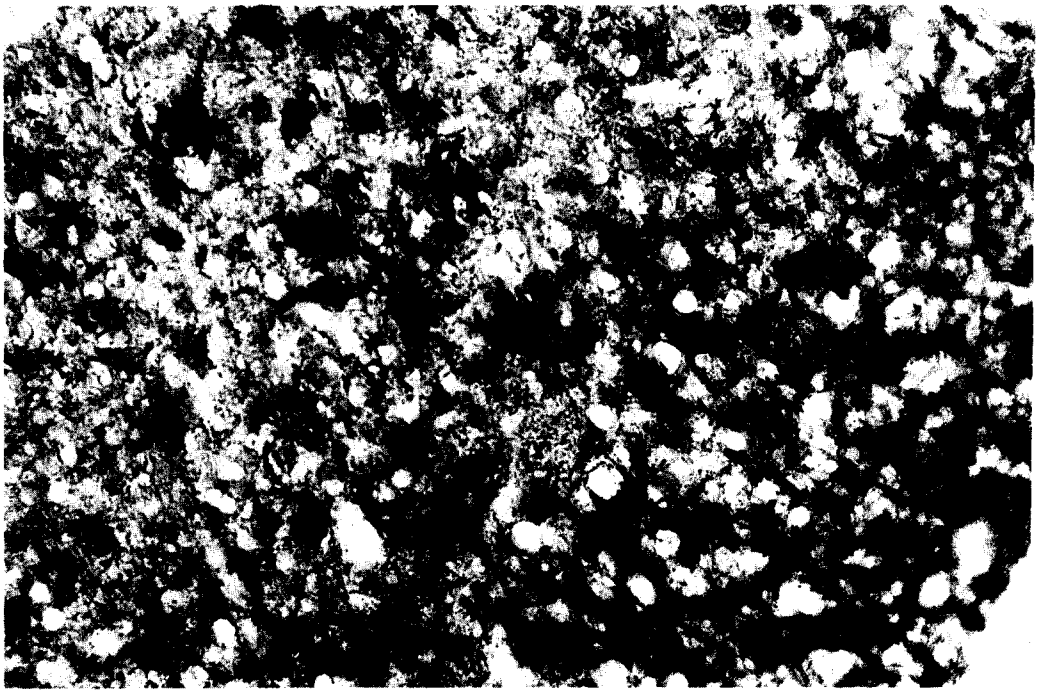

FIG. 5. 16K positive cells in the arcuate nucleus. These same cells are positive for all other 31K antisera. ×155.

of 31K (16K). The net effect of these release studies appears to argue that in humans, at least, major portions of this brain peptide system are electrically releaseable and would theoretically be biologically active.

A number of opiate receptor studies using specifically labelled beta-endorphin have demonstrated high affinity binding sites in central nervous system [1, 20, 24]. Such studies, along with others using beta-endorphin as a competitive inhibitor against other opiate ligands, suggest that beta-endorphin binds to both the delta receptor (which is selective for enkephalin) and the mu receptors (selective for morphine) [10,27]. More detailed studies (currently under-

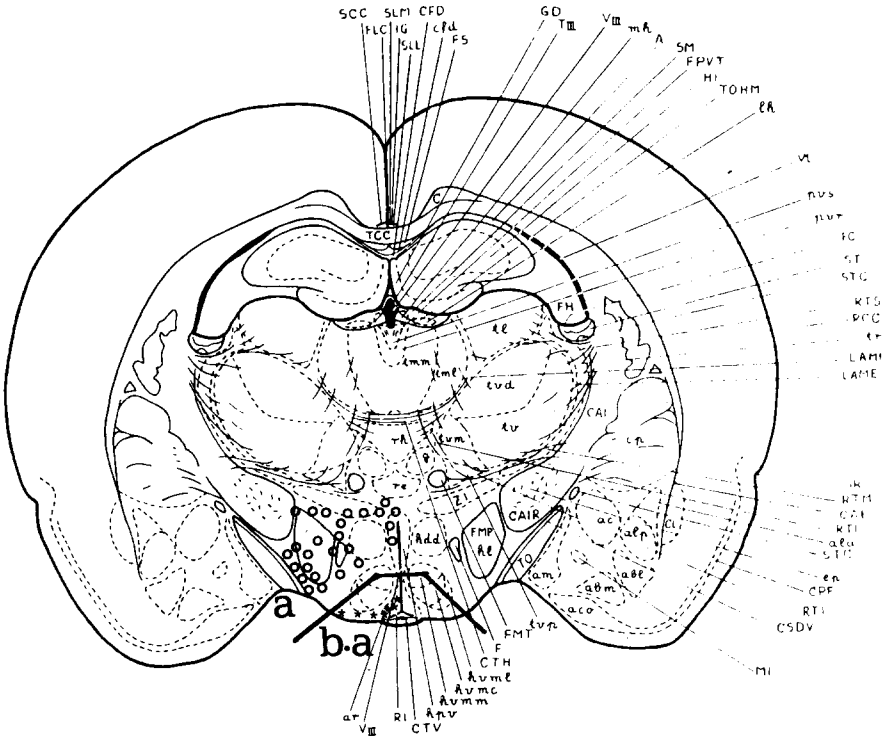

FIG. 6. Schematic in coronal section through rat hypothalamus. The stars within the medial-inferior line with the notation "b.a" indicate arcuate 31K cells (α-MSH, β-END, 16K, etc). The open circles above the line "a" indicate the cells staining for α-MSH only. Adapted from Konig and Klippel's atlas.

way) are necessary to determine whether beta-endorphin labels a subpopulation of opiate receptors unique to this opiate peptide (Hewlett, Akil, Barchas, and Li).

There have been relatively fewer studies of alpha-MSH and gamma-MSH or ACTH in receptors in central nervous system. To date these studies have been extremely difficult. Our experience with them (in collaboration with J. Ramachandran, U.C.S.F.) has revealed the existence of saturable binding of labelled ACTH and alpha-MSH to central nervous system membranes. However, the results are variable from experiment to experiment possibly due to the great hydrophobic nature of these peptides. Our results generally suggest the existence of a small number of ACTH and alpha-MSH binding sites, which are differentially distributed across limbic structures.

If one shifts to microinjection of ACTH, alpha-MSH, gamma-MSH and evaluates the effects of the pharmacological administration of these peptides a most interesting problem emerges. As is well known the administration of opiates and opiate peptides can produce a narcotic specific analgesia when injected into the central gray area of rats (cf [47]). Using the same paradigm it has been possible to demonstrate that ACTH, alpha-MSH, des-acetyl-alpha-MSH and Organon 2766 (an active analog of MSH) all produce a potent analgesia [47]. The rank ordering places the degradation resistant Organon analog at the top of the list with alpha-MSH being least potent. The Org 2766 compound is as potent as morphine in producing an analgesia. It should be noted that this analgesia is not cross tolerant with morphine nor is it blockable by naloxone. It would appear from these studies that not only is analgesia produced by the opiate portion of the 31K molecule, but that other portions of that same molecule appear to be capable of producing a significant biological action in the same direction (a non-opiate analgesia). Of further interest is the fact that the MSH/ACTH analgesia is most potent in the region of the beta endorphin bundle (and 31K product receptors). The net effect of these studies is to suggest that the 31K molecule is designed to produce a set of actions—some of which are opiate mediated, some not, but all of which are in the same physiological directions. Thus one would expect an opiate analgesia as well as a non-opiate analgesia acting in concert. But what of the newly discovered gamma-MSH [30]? Does it produce an opiate-like or non-opiate-like analgesia? Does it have any actions at all? When injected in approximately equal molar amounts gamma-MSH 1–12 does not appear to produce an ACTH, alpha-MSH or Org 2766-like analgesia. In fact no obvious effect can be seen for the administration of this agent. However, when it is administered in concert with ACTH, gamma-MSH is found to substantially potentiate the peak analgesia obtained from ACTH. It is as though the role of gamma-MSH is to provide another action which potentiates that of ACTH. This same sort of gamma-MSH/ACTH potentiation has been observed by others in the production of steroids from adrenal cells [33]. While these data are preliminary and in some ways difficult to interpret, perhaps the most reasonable suggestion would be that the net effect on the post-synaptic element of all the 31K products (beta-endorphin, alpha-MSH or ACTH and gamma-MSH) would be a very powerful addition or synergism produced by a variety of mechanisms. On the other hand, ACTH and beta-END have been suggested to be mixed against/an-

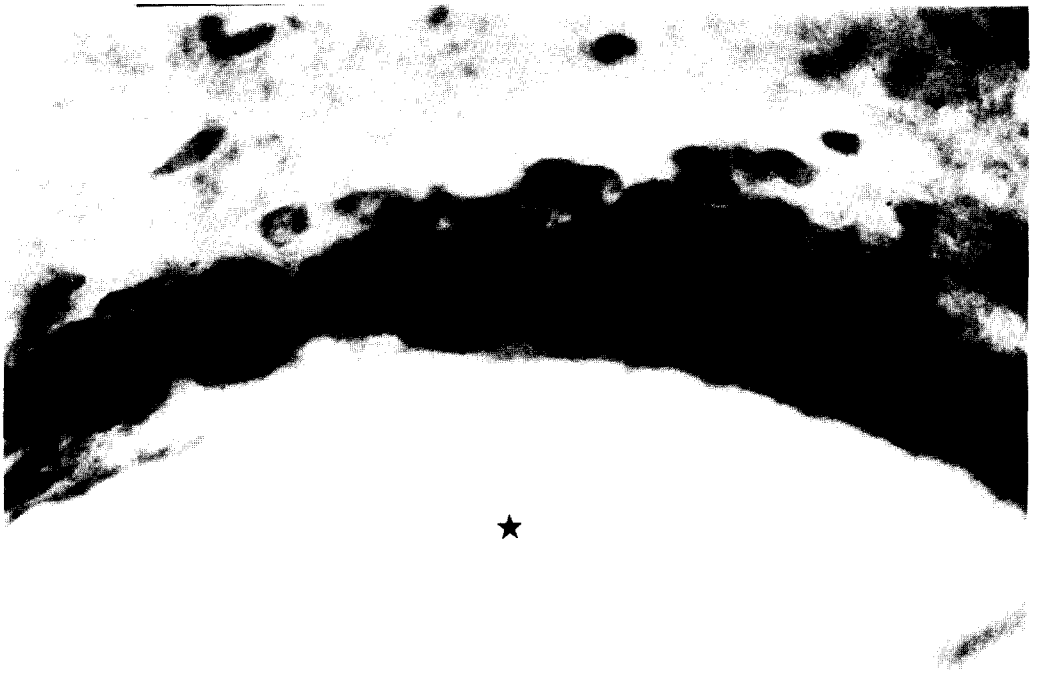

FIG. 7. A large group of dynorphin positive cells in the supraoptic nucleus (star in the optic tract). This is a 50 μm thick section. $\times$540.

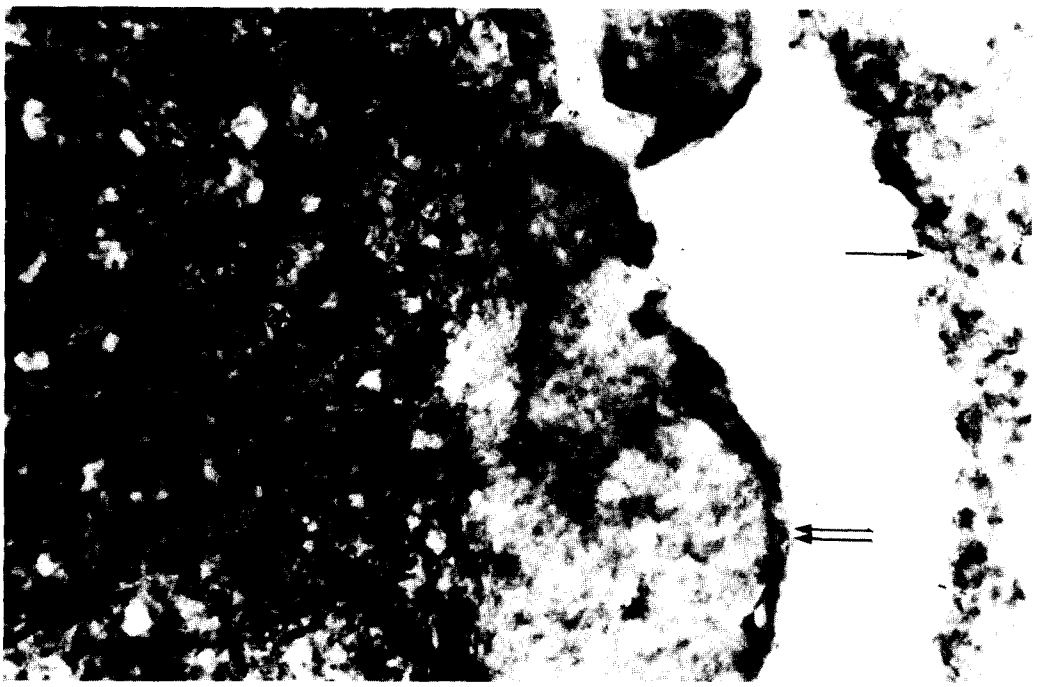

FIG. 8. Dynorphin positive processes in the posterior pituitary lobe (star), but not in the anterior (one arrow) or intermediate lobe (two arrows). $\times$300.

tagonist in other paradigms. Thus, the specific interactions and regulation of multiple substances deriving from pro-opiocortin proves to be new areas for research.

The Enkephalins and Dynorphin: Related Substances or Not?

The enkephalins were the first opiate peptides discovered [21] and in many ways have been much better studied than the beta-endorphin system. However, much less information is known about certain aspects of the enkephalins. For example, relatively little is known about the specific distribution of methionine versus leucine enkephalin, or the biosynthesis of enkephalins. In contrast a great deal is known about their structure activity relationships, their re-

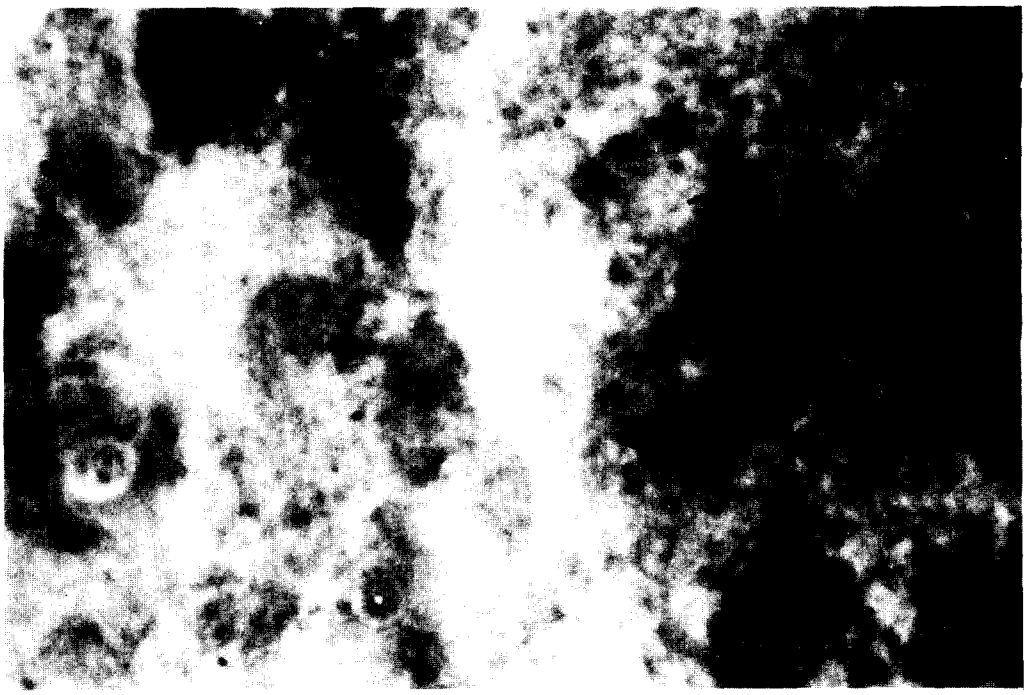

FIG. 9. Dynorphin positive axon (arrows) in the ventromedial hypothalamic nucleus. ×2100.

ceptors and their pharmacology. There are few obvious facts relevant to this paper.

The enkephalins have been found to be separate from beta-endorphin [6,52] even though methionine-enkephalin shares a common sequence portion with the N terminus of beta-endorphin [21]. The distribution of the enkephalins (both methionine- and leucine-enkephalins) has been widely studied in rodent central nervous system [11, 13, 19, 41, 44, 45, 48, 53]. A large number of cell groups has been discovered, scattered from rostral limbic structures to spinal cord (not to mention adrenal, peripheral nerves and ganglia) [42,43]. The enkephalin systems are thus extremely widespread, having extensive fiber distributions locally and in some cases, projecting along well defined pathways to neighboring structures, as in the case of the pallido-striatal enkephalin pathway [11]. Their anatomy suggests that enkephalins are well situated for major involvement in most of the functions classically associated with opiate action, such as respiration, temperature or pain control. A significant question relating to the distribution of methionine- and leucine-enkephalin revolves around whether they were in the same or different cells. Recent evidence by Larsson and co-workers [23] tends to strongly suggest that methionine- and leucine-enkephalin in every system studied to date are in fact separable systems. While the distribution of these two systems is extremely similar in the gross anatomical sense, careful study with properly prepared antisera allows one to see differences between the distribution of methionine- and leucine-enkephalin cells. Thus it is possible to raise questions about separate physiological function and even separate receptors. The question of multiple receptors is extremely complicated, as many opiate alkaloids and peptides are rather "indiscriminant" in the subtype of receptors they can bind to [10]. However, it has recently become possible to suggest that methionine- and leucine-enkephalin bind with somewhat different affinities to the different subtypes of opiate receptors with leucine-enkephalin being more prototypical of the delta receptor, and met-enkephalin exhibiting somewhat more reactivity to the mu site. Nevertheless, both peptides are more delta-like than is beta-endorphin, which interacts with mu sites and delta sites with great ease [10,27].

Adding to the confusion of the relationship between methionine- and leucine-enkephalin is the question of the biosynthesis of the enkephalins. Relatively little progress has been made on specific biosynthesis of the enkephalins of central nervous system (in contrast the beta-endorphin). However, recent work in adrenal by Lewis and co-workers [25] strongly suggests that in, at least, adrenal the enkephalins come from a 55,000 Dalton precursor which contains seven replicates of methionine-enkephalin and one replicate of leucine-enkephalin. Whether this same biosynthetic pattern will be found in central nervous system and how that will relate to the apparent separate distribution of methionine-leucine-enkephalin is an open question at this point.

Most recently another opiate peptide has been discovered by Avram Goldstein and co-workers at Stanford [16]. This substance, known as dynorphin, was purified from pig pituitary using a guinea pig ileum bioassay. In analyzing the structure of dynorphin it became apparent that dynorphin contained the sequence of leucine-enkephalin as its N-terminus. However, the C terminus of dynorphin (as far as it is known) contains eight amino acids which are unique in that they do not appear to occur in any other known opiate or non-opiate peptide. This substance would appear to have the same type of relationship to leucine-enkephalin as methionine-enkephalin does to beta-endorphin. And the same types of questions have arisen about it. Where is dynorphin? How is it synthesized? What is its relationship to the enkephalins, and to other peptides?

Using immunocytochemical techniques we (in collaboration with Avram Goldstein and co-workers) [51] have been

able to locate dynorphin in the magnocellular neurons of hypothalamus (supraoptic nucleus, paraventricular nucleus) with fiber projections to the posterior pituitary. For technical reasons it has been difficult to demonstrate other dynorphin cell bodies and fiber in central nervous system. However, extensive blocking studies in brain and pituitary have allowed the conclusion that dynorphin is separable from leucine-enkephalin in the supraoptic nucleus and posterior pituitary. Biochemical studies using HPLC and RIA [14] have strongly reinforced the existence of both peptides in posterior pituitary [51]. Dynorphin and leu-enkephalin appear to have quite different distributions in the posterior lobe, with pituitary dynorphin being contained in large neurosecretory processes [51] and endings whereas enkephalin is contained within axonal-like fibers in the portion of the gland near the intermediate lobe [40]. It is, nevertheless, possible that both opioids arise from the same cells of origin (and even possibly the same precursor protein) but are stored and transported via different pituitary procedures. It is known that vasopressin, oxytocin and neurophysin are contained in the same general type of magnocellular neurons (cf [56]). However, it is not clear whether dynorphin is in these cells or perhaps other magnocellular cells in the same general nucleus.

Thus, with respect to both enkephalin and dynorphin, several substantial questions continue to exist. Most critical among those is whether they arise from a common cell of origin, or share a common biosynthetic route, and whether they constitute anatomically and physiologically separate systems, with distinct receptor populations.

Conclusion

It can be seen from the above overview that the opioid peptide systems have opened up new avenues of thought in neuroscience research. The existence of multiple opioid and non-opioid peptides within the same cells (beta-endorphin/alpha-MSH/gamma-MSH) is a particularly important phenomenon since it suggests the possibility that they might act in concert to modulate physiology–as indicated by recent findings. Furthermore, the possibility that the same cell may produce two opioids—methionine- and leucine-enkephalin in adrenal and brain or leucine-enkephalin and dynorphin in hypothalamus—also raises several interesting questions with regard to their combined roles. On the other hand, if these related peptides—eg enkephalins and dynorphin—are found in separate anatomical pathways and bind to different receptors—the redundancy of peptide structure and opiate function becomes a question of great import.

In all cases, the study of endogenous opioids sheds light not only on these systems per se, but on more general principles of neurobiology.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to P. Erskine for manuscript preparation and R. Thompson and S. Burke for laboratory assistance.

REFERENCES

1. Akil, H., W. Hewlett, J. D. Barchas and C. H. Li. Binding of 3H-β-endorphin to rat brain membranes: Characterization and opiate properties. *Eur. J. Pharmac.* **64:** 1–8, 1980.
2. Akil, H., D. E. Richardson, J. D. Barchas and C. H. Li. Appearance of β-endorphin-like immunoreactivity in human ventricular cerebrospinal fluid upon analgesic electrical stimulation. *Proc. natn. Acad. Sci. U.S.A.* **75:** 5170–5172, 1978.
3. Akil, H. and S. J. Watson. Neuromodulatory functions of the brain pro-opiocortin system. In: *Advances in Biochemical Psychopharmacology,* edited by M. Trabucchi and E. Costa. New York: Raven Press, 1980, pp. 435–445.
4. Bloch, B., C. Bugnon, D. Fellman and D. Lenys. Immunocytochemical evidence that the same neurons in the human infundibular nucleus are stained with anti-endorphins and antisera of other related peptides. *Neurosci. Lett.* **10:** 147–152, 1978.
5. Bloom, F. Behavioral implications of anterior pituitary hormones found in brain. *Soc. Neurosci. Abstr.* **5:** 103, 1979.
6. Bloom, F. E., E. Battenberg, J. Rossier, N. Ling and R. Guillemin. Neurons containing β-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. *Proc. natn. Acad. Sci. U.S.A.* **75:** 1591–1595, 1978.
7. Bloom, F. E., E. Battenberg, J. Rossier, N. Ling, J. Leppaluoto, T. M. Vargo and R. Guillemin. Endorphins are located in the intermediate and anterior lobes of the pituitary gland, not in the neurohypophysis. *Life Sci.* **20:** 43–48, 1977.
8. Bloom, F. E., J. Rossier, E. L. F. Battenberg, A. Bayon, E. French, S. J. Henricksen, G. R. Siggins, D. Segal, R. Browne, N. Ling and R. Guillemin. β-Endorphin: cellular localization, electrophysiological and behavioral effects. In: *The Endorphins: Advances in Biochemical Psychopharmacology, Vol. 18,* edited by E. Costa and M. Trabucchi. New York: Raven Press, 1978, pp. 89–109.
9. Bradbury, A. F., W. F. Feldberg, D. G. Smyth and C. Snell. Lipotropin C-fragment: An endogenous peptide with potent analgesic activity. In: *Opiates and Endogenous Opioid Peptides,* edited by H. W. Kosterlitz. Amsterdam: North Holland Publishing Co., 1976, pp. 9–17.
10. Chang, J. J., B. R. Cooper, E. Hazum and P. Cuatrecasas,. Multiple opiate receptors: Different regional distribution in the brain and differential binding of opiates and opioid peptides. *Molec. Pharmac.* **16:** 91–104, 1979.
11. Cuello, A. C. and G. Paximos. Evidence for a long leu-enkephalin striopallidal pathway in rat brain. *Nature* **271:** 178–180, 1978.
12. Eipper, B. and R. Mains. Existence of a common precursor to ACTH and endorphin in the anterior and intermediate lobes of the rat pituitary. *J. Supramolec. Struct.* **8:** 247–262, 1978.
13. Elde, R., T. Hokfelt, O. Johansson and L. Terenius. Immunohistochemical studies using antibodies to leucine enkephalin: Initial observations on the nervous system of the rat. *Neuroscience* **1:** 349–351, 1976.
14. Ghazarossian, V. E., C. Chavkin and A. Goldstein. A specific radioimmunoassay for the novel opioid peptide dynorphin. *Life Sci.* **27:** 75–86, 1980.
15. Gianoulakis, D., N. G. Seidah, R. Routhier and M. Chretien. "*In vitro* biosynthesis and chemical characterization of ACTH and ACTH fragments by the rat pars intermedia." In: *Endogenous and Exogenous Opiate Agonists and Antagonists,* edited by E. Leong Way. New York: Pergamon Press, 1980, pp. 289–292.
16. Goldstein, A., S. Tachibana, L. I. Lowney, M. Hunkapiller and L. Hood. Dynorphin-(1–13), an extraordinarily potent opioid peptide. *Proc. natn. Acad. Sci. U.S.A.* **76:** 6666–6670, 1979.

17. Guillemin, R., N. Ling and R. Burgus. Endorphins, peptides d'origine hypothalamique and neurohypophysaire d'activite morphinomimetique. Isolement et structure moleculaire d'alpha-endorphine. *C. r. hebd. Séanc. Acad. Sci. Paris* **282:** 783–785, 1976.
18. Hosobuchi, Y., J. Rossier and F. Bloom. Oral loading with L-tryptophan may augment the simultaneous release of ACTH and β-endorphin that accompanies periaqueductal stimulation in humans. In: *Neural Peptides and Neuronal Communication,* edited by E. Costa and M. Trabucchi. New York: Raven Press, 1980, pp. 563–570.
19. Hokfelt, T., R. Elde, O. Johansson, L. Terenius and L. Stein. The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. *Neurosci. Lett.* **5:** 25–31, 1977.
20. Houghton, R. A. and C. H. Li. Preparation and properties of tritiated human β-endorphin with high specific radioactivity. *Int. J. Peptide Prot. Res.* **12:** 325–326, 1978.
21. Hughes, J., T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan and H. R. Morris. Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* **258:** 577–579, 1975.
22. Krieger, D. T., A. Liotta and M. J. Brownstein. Presence of corticotropin in brain of normal and hypophysectomized rats. *Proc. natn. Acad. Sci. U.S.A.* **74:** 648–652, 1977.
23. Larsson, L. I., S. Childers and S. H. Snyder. Methionine and leucine enkephalin occur in separate neurons. *Nature* **22:** 407–410, 1979.
24. Law, P. Y., R. A. Houghten, H. H. Loh and C. H. Li. Characterization of a high affinity ^{3}H-βh-endorphin receptor in rat brain crude synaptosomal fraction. In: *Endogenous and Exogenous Opiate Agonists and Antagonists,* edited by E. Leong Way. New York: Pergamon Press, 1980, pp. 225–228.
25. Lewis, R. V., A. S. Stern, S. Kimura, J. Rossier, S. Stein and S. Udenfriend. On about 50,000 dalton protein in adrenal medulla: A common precursor of (met) and (leu) enkephalin. *Science* **208:** 1459–1461, 1980.
26. Li, C. H. and D. Chung. Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. *Proc. natn. Acad. Sci. U.S.A.* **73:** 1145–1148, 1976.
27. Lord, J. A. H., A. A. Waterfield, J. Hughes and H. W. Kosterlitz. Multiple opiate receptors. In: *Opiates and Endogenous Opioid Peptides,* edited by H. W. Kosterlitz. Amsterdam: Elsevier/North Holland Press, 1976, pp. 275–280.
28. Mains, R. E., B. A. Eipper and N. Ling. Common precursor to corticotropins and endorphins. *Proc. natn. Acad. Sci. U.S.A.* **74:** 3014–3018, 1977.
29. Moon, H. D., C. H. Li and B. M. Jennings. Immunohistochemical and histochemical studies of pituitary β-lipotropin. *Anat. Rec.* **175:** 524–538, 1973.
30. Nakanishi, S., A. Inoue, T. Kita, M. Nakamura, A. C. Y. Chang, S. N. Cohen and S. Numa. Nucleotide sequence of cloned cDNA for bovine corticotropin-beta-lipotropin precursor. *Nature* **278:** 423–427, 1979.
31. Nilaver, G., E. A. Zimmerman, R. Defendini, A. Liotta, D. A. Krieger and M. Brownstein. Adrenocorticotropin and β-lipotropin in hypothalamus. *J. Cell Biol.* **81:** 50–58, 1979.
32. O'Donohue, T. and D. Jacobowitz. Recent studies of α-melanotropinergic nerves in the brain. Presented at the *International Society of Psychoneuroendocrinology,* August 8–11, Salt Lake City, 1979.
33. Pedersen, R. C., A. C. Brownie and N. Ling. Pro-adrenocorticotropin/endorphin-derived peptides: coordinate action on adrenal steroidogenesis. *Science 1208,* (198), 1044–1046.
34. Pelletier, G. Ultrastructural localization of a fragment (16K) of the common precursor for adrenocorticotropin (ACTH) and β-lipotropin (β-LPH) in the rat hypothalamus. *Neurosci. Lett.* **16:** 85–90, 1980.
35. Pelletier, G. and D. Dube. Electron microscopic immunohistochemical localization of α-MSH in the rat brain. *Am. J. Anat.* **150:** 201–206, 1977.
36. Pelletier, G., R. Leclerc, F. Labrie, J. Cote, M. Chretien and M. Les. Immunohistochemical localization of β-lipotropin hormone in the pituitary gland. *Endocrinology* **100:** 770–776, 1977.
37. Richardson, D. E. and H. Akil. Pain reduction by electrical stimulation in man: Part 1: Acute administration in periaqueductal and periventricular sites. *J. Neurosurg.* **47:** 178–183, 1977.
38. Richardson, D. E. and H. Akil. Pain reduction by electrical brain stimulation in man. Part 2: Chronic self-administration in the periventricular gray matter. *J. Neurosurg.* **47:** 184–194, 1977.
39. Roberts, J. L. and E. Herbert. Characterization of a common precursor to corticotropin and β-lipotropin: identification of β-lipotropin peptides and their arrangement relative to corticotropin in the precursor synthesized in a cell-free system. *Proc. natn. Acad. Sci. U.S.A.* **74:** 5300–5304, 1977.
40. Rossier, J., E. Battenberg, Q. Pittman, A. Bayon, L. Koda, R. Miller, R. Guillemin and F. Bloom. Hypothalamic enkephalin neurons may regulate the neurohypophysis. *Nature* **277:** 653–655, 1979.
41. Sar, M., W. E. Stumpf, R. J. Miller, K-J. Chang and P. Cuatrecasas. Immunohistochemical localization of enkephalin in rat brain and spinal cord. *J. comp. Neurol.* **182:** 17–37, 1978.
42. Schultzberg, M., C. F. Dreyfus, M. D. Gershon, T. Hokfelt, R. Elde, G. Nilsson, S. Said and M. Goldstein, VIP-, enkephalin-, substance P-, and somatostatin-like immunoreactivity in neurons intrinsic to the intestine: Immunohistochemical evidence from organotypic tissue cultures. *Brain Res.* **155:** 239–248, 1978.
43. Schultzberg, M., J. M. Lundberg, T. Hokfelt, L. Terenius, J. Brandt, R. P. Elde and M. Goldstein. Enkephalin-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. *Neuroscience* **3:** 1169–1186, 1979.
44. Simantov, R., M. J. Kuhar, G. R. Uhl and S. H. Snyder. Opioid peptide enkephalin: immunohistochemical mapping in rat central nervous system. *Proc. natn. Acad. Sci. U.S.A.* **74:** 2167–2171, 1977.
45. Uhl, G. R., M. J. Kuhar and S. H. Snyder. Enkepahlin containing pathway amygdaloid efferents in the stria terminalis. *Brain Res.* **149:** 223–228, 1978.
46. VanLeeuwen, F. W., D. F. Swaab, C. deRaay and B. Fisser. Immunoelectron-microscopical demonstration of α-melanocyte-stimulating hormone-like compound in the rat brain. *J. Endocr.* **80:** 59P–60P, 1979.
47. Walker, J. M., H. Akil and S. J. Watson. Analgesic effects of α-MSH and related peptides: Evidence for homologous actions of pro-opiocortin products. *Science,* in press.
48. Walmsley, J. K., W. S. Young and M. J. Kuhar. Immunocytochemical localization of enkephalin in rat forebrain. *Brain Res.* **190:** 153–174, 1980.
49. Watson, S. J. and H. Akil. α-MSH in rat brain: Occurrence within and outside brain β-endorphin neurons. *Brain Res.* **182:** 217–223, 1980.
50. Watson, S. J. and H. Akil. The presence of two α-MSH positive cell groups in rat hypothalamus. *Eur. J. Pharmac.* **58:** 101–103, 1980.
51. Watson, S. J., H. Akil, V. E. Ghazarossian and A. Goldstein. Dynorphin immunocytochemical localization in brain and peripheral nervous system. Submitted.
52. Watson, S. J., H. Akil, C. W. Richard and J. D. Barchas. Evidence for two separate opiate peptide neuronal systems and the coexistence of β-lipotropin, β-endorphin, and ACTH immunoreactivities in the same hypothalamic neurons. *Nature* **275:** 226–228, 1978.
53. Watson, S. J., H. Akil, S. O. Sullivan and J. D. Barchas. Immunocytochemical localization of methionine-enkephalin: preliminary observations. *Life Sci.* **25:** 733–738, 1977.
54. Watson, S. J., J. D. Barchas and C. H. Li. β-Lipotropin: localization of cells and axons in rat brain by immunocytochemistry. *Proc. natn. Acad. Sci. U.S.A.* **74:** 5155–5158, 1977.

55. Watson, S. J., C. W. Richard and J. D. Barchas. Adrenocorticotropin in rat brain: immunocytochemical localization in cells and axons. *Science* **200:** 1180–1182, 1978.
56. Zimmerman, E. A. Localization of hypothalamic hormones by immunocytochemical techniques. In: *Frontiers in Neuroendocrinology 4*, edited by L. Martini and W. F. Ganong. New York: Raven Press, 1976, pp. 25–62.
57. Zimmerman, E. A., A. Liotta and D. T. Krieger. β-Lipotropin in brain: Localization in hypothalamic neurons by immunoperoxidase technique. *Cell Tiss. Res.* **186:** 393–398, 1978.