60 YEARS OF POMC
Lipotropin and beta-endorphin: a perspective

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Abstract

Many important fields of research had a humble origin. In the distant past, A J P Martin’s discovery that amino acids could be separated by paper chromatography and Moore and Stein’s use of columns for quantitative amino acid analysis provided the first steps towards the determination of structure in complex biologically active molecules. They opened the door to reveal the essential relationship that exists between structure and function. In molecular endocrinology, for example, striking advances have been made by chemists with their expertise in the identification of structure working with biologists who contributed valuable knowledge and experience. Advantage was gained from the convergence of different background, and it is notable that the whole is greater than the sum. In the determination of structure, it may be recalled that four of the world’s great pioneers (Archibald Martin, Rodney Porter, Fred Sanger and Vincent du Vigneaud) were acknowledged for their fundamental contributions when individually they were awarded the Nobel Prize. They foresaw that the identification of structure would prove of outstanding importance in the future. Indeed, study of the structures of β-endorphin and enkephalin and the different forms of opiate activity they engender has led to a transformation in our understanding of chemical transmission in the brain.

Key Words
lipotropin
neurotransmitters
beta-endorphin
POMC

Lipotropin: precursor of β-endorphin

It is well known, of course, that most if not all peptide hormones are formed as fragments of larger peptides and lipotropin is no exception. This 91-residue polypeptide, first isolated by Birk and Li (1964) and sequenced by Li et al. (1965), possessed lipolytic activity, and it was provisionally assigned the title ‘lipotrophic hormone’. The primary structure included a sequence of amino acids that exhibited a marked similarity to α-melanotropin (α-MSH), and it was generally assumed that ‘β-MSH’ was the biologically active counterpart to α-MSH in lipotropin. As a melanotrophic hormone, however, β-MSH was much less active than α-MSH and alarm bells began to ring when it was reported that β-MSH was not present in the human pituitary (Bloomfield et al. 1974), which raised question on its physiological significance. This set the stage for two unexpected discoveries: firstly, that a 31-residue fragment of lipotropin shown to be present in pituitary and brain possessed potent opiate activity (Feldberg & Smyth 1976, Bradbury et al. 1976a, Li & Chung 1976); and secondly, that lipotropin was itself a fragment of a precursor, which proved to be a multifunctional prohormone (Mains et al. 1977, Roberts & Herbert 1977, Nakanishi et al. 1979). It is of interest to recall the background to these advances which stemmed from the study of prohormone processing and generation of their biologically active constituents.

Lipotropin: from structure to function

For several years, the principal interests of my laboratory at the National Institute for Medical Research
(Mill Hill, London) were focused on β-endorphin and its formation from lipotropin. Donald Steiner’s classical studies on insulin precursors had demonstrated that insulin is formed from a prohormone, and it seemed likely that this would prove to be a general phenomenon. As fragments of prohormones might be retained in tissues where the prohormones are synthesised, our strategy was to isolate and identify the constituent fragments of a putative prohormone and then reassemble them to reveal its linear structure. In 1974, we were successful in isolating a series of novel peptides from porcine pituitary (Bradbury et al. 1975, 1976b) and we observed that a group of them accounted for the sequence of adrenocorticotrophic hormone (ACTH) together with the sequence of lipotropin. At that time, it was not known that ACTH and lipotropin are formed from a common prohormone, although the possibility had been discussed informally at Meetings of the Endocrine Society (see Lowry 2016).

The peptides we isolated could have been inert fragments ‘left over’ after activation of a prohormone, or they might possess an activity that was waiting to be discovered. To obtain an indication of this, we examined the sensitivity of lipotropin to cleavage by trypsin and also by an enzyme with similar specificity which we had isolated from the pituitary, because it was believed that peptide hormones were generated from their precursors by enzymes with basic specificity (Smyth 1975). We found that one of the peptides we identified in the pituitary could be released from the C-terminus of lipotropin by limited digestion with either enzyme (Bradbury et al. 1976c), but surprisingly there was negligible cleavage on the N-terminal side of the sequence at the centre of lipotropin necessary for the release of β-MSH, which at the time was believed to be the biologically active region.

This implied that the C-terminal peptide rather than β-MSH might be functionally significant, so I arranged a collaborative study with James Edwardson of Imperial College, London, to investigate its activity. As the C-terminal peptide 1, named ‘C-fragment of lipotropin’, had been isolated from the pituitary which secretes its hormones into the circulation, Edwardson started by investigating potential peripheral activities such as effects on sodium transport in the kidney and influence on insulin secretion from the pancreas.

**Discovery of β-endorphin**

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In discussion with Howard Morris after my lecture, it became apparent that the four residues at the N-terminus of the Leu-enkephalin and Met-enkephalin peptides which had been identified corresponded to this N-terminal sequence of the C-fragment of lipotropin, pointing to a structural and possibly functional relationship between these peptides.

Morris told me that he was involved in an important but confidential collaboration which he was not free to discuss, but the next morning Hans Kosterlitz, the head of a pharmacology group in Aberdeen, Scotland, telephoned to let me know what the secrecy was about.

Sir Arnold Burgen, the director of the National Institute for Medical Research (NIMR), Mill Hill, London, at once arranged a meeting with Kosterlitz and two of his colleagues, John Hughes and Howard Morris, and myself, to take place in his office at the NIMR. A few days later, Kosterlitz and Hughes came down from Aberdeen to London for the meeting, and we discussed the background and progress made in their identification of the enkephalins and our investigation of the C-fragment of lipotropin. It was clear that there was an unexpected but highly significant overlap in our research, so it was
decided that Kosterlitz and Hughes who were funded in part by the Medical Research Council (MRC) (as was the NIMR) should continue their work by concentrating on receptors for the enkephalins, while we would investigate the physiological role of the C-fragment (our peptide was later renamed ‘β-endorphin’ by Eric Simons and C H Li). Thus, at that time we had isolated and identified β-endorphin and a related 27 residue peptide, and in collaboration with Edwardson we were searching for the biological activity of β-endorphin.

Looking back, it is clear that our work on the processing of lipotropin which led us to isolate the C-fragment (β-endorphin) and the work of the Kosterlitz group from Aberdeen on the identification of the enkephalins (Hughes et al. 1975) had converged by serendipity. In any case, it is abundantly clear that identification of enkephalin and β-endorphin opened up an exciting area of brain biochemistry.

Li and his colleagues prepared β-endorphin by the method of solid-phase synthesis and they reported that the 31-residue peptide exhibited strong analgesic activity (Loh et al. 1976). Using the hot plate assay performed on the rat, it was demonstrated that β-endorphin was much more potent than morphine. Li isolated β-endorphin from the pituitary of the camel (Li & Chung 1976) and the ox. Thus, Li’s achievement in determining the sequence of lipotropin 10 years before (Li et al. 1965) and his further isolation and characterisation of γ-lipotropin (Chretien & Li 1967) led on to the identification of β-endorphin.

**β-endorphin and opiate activity**

With Birdsay and Hulme, we demonstrated that the C-fragment of lipotropin (β-endorphin) has a high affinity for opiate receptors in the brain, and the binding was reversed by naloxone, a classical antagonist of the opiates (Bradbury et al. 1976a). Alongside this, Feldberg found that β-endorphin administered in cat ventricles was 100 times more potent than morphine as an analgesic agent (Feldberg & Smyth 1976, 1977) and the analgesia persisted for several hours. Feldberg concluded that β-endorphin was the most potent analgesic agent known.

In addition to its analgesic properties, Feldberg observed that β-endorphin produced a range of other profound central effects characteristic of morphine. We had hoped that as it occurs naturally, β-endorphin would not produce tolerance and dependence, but experiments showed that this was not to be (Gispen et al. 1976, Van Ree et al. 1976, 1979, Jacquet et al. 1978). With Deakin and Wendlandt, we went on to show that β-endorphin was highly potent in the rat (Bradbury et al. 1977), but little or no analgesic effects could be detected with the natural enkephalins (Smyth 1976, Bradbury et al. 1977). The enkephalins act at δ-opioid receptors, and their half-lives in brain are only a few seconds. It should be noted, however, that the activity of the enkephalins can be demonstrated unequivocally in opiate assays in vitro.

The striking difference between the long-lasting actions of β-endorphin and the transient effects of the enkephalins might be due to resistance exhibited by β-endorphin to degradation by proteolytic enzymes in the brain. At the structural level, the stability of the 31-residue peptide could be attributed to its tendency to adopt a conformation that would make it less vulnerable to enzymatic attack. We obtained evidence for this by digesting methionine enkephalin and β-endorphin with leucine aminopeptidase, an enzyme that removes amino acids from the NH₂ terminus. We also digested the two peptides with a membrane-bound aminopeptidase from rat brain. In both cases, under conditions where the enkephalin was rapidly degraded, β-endorphin which has the same N-terminal sequence as enkephalin remained stable (Fig. 2) (Austen & Smyth 1977, Austen et al. 1979).

In contrast to the high potency of β-endorphin applied in the ventricles, very weak or no analgesic activity was obtained by intravenous administration. This implied that it is only in the brain that β-endorphin can participate in analgesic mechanisms: the pituitary peptide does not appear to have access to the analgesic receptors. In studying pain mechanisms (Smyth 1983), it is important therefore to determine the levels of β-endorphin in the regions of brain that are involved in analgesia or to determine the levels in the cerebrospinal fluid (CSF). It is also important, of course, to establish that the peptide is present in its biologically active form.

**β-endorphin in the brain**

Immunohistochemical staining of rat tissue sections showed that the principal areas where fluorescence was seen were the hypothalamus, thalamus-mid brain, amygdala, hippocampus and brainstem (Smyth & Zakarian 1982, Zakarian & Smyth 1982a,b) (Fig. 3). Dense fluorescence was also observed in the region of the dorsal colliculi. The immunoreactivity in the hypothalamus was confined to the arcuate nucleus, the median eminence and the ventromedial border of the third ventricle, but axons and terminals could also be seen along the walls of the third ventricle. From the hypothalamus, long beaded axons extended...
dorsally and laterally, the fluorescence becoming less dense in the thalamus and terminals were present at more distant locations in the amygdala, colliculi and hippocampus. In general, immunofluorescence revealed a highly organised network commencing with the hypothalamic cell bodies, ramifying through long axons and ending in bundles of terminals at a number of defined locations.

Gel exclusion of β-endorphin-related peptides extracted from regions of rat brain showed that lipotropin was only a minor component; the majority of the peptides had the approximate molecular size of β-endorphin. The β-endorphin-related peptides were identified by extraction from the tissues and ion-exchange chromatography, the amounts being determined by radioimmunoassay (Smyth & Zakarian 1982, Zakarian & Smyth 1982b, Snell et al. 1977). Two distinct processing patterns were apparent: the first was characteristic of the hypothalamus, midbrain and amygdala, and the second of the hippocampus, brainstem and colliculi (Fig. 4). The hypothalamus contained predominantly β-endorphin(1–31), and
this was the major β-endorphin-related peptide in the midbrain and amygdala but accompanied there by β-endorphin(1–27) and β-endorphin(1–26); in these regions, there were negligible concentrations of acetylated peptides. In contrast, the hippocampus, brainstem and colliculi contained principally N-acetyl forms of β-endorphin(1–27) and β-endorphin(1–26) which are devoid of opiate activity.

The results showed that the nerve terminals in certain regions of the brain store the potent form of β-endorphin and it is possible that the physiological functions associated with these regions may involve regulation by opiate activity. In the regions of brain where the inactive forms of β-endorphin predominated, opiate-controlled neurotransmission would seem to be a less common phenomenon. It is significant, however, that the cells that contained C-terminally shortened forms of β-endorphin would also contain glycyl-glutamine released from the C-terminus of the 31-residue peptide (Parish & Smyth 1982). In a related electrophysiological study, Wolstencroft demonstrated that this dipeptide inhibited the firing of brainstem neurons (Parish et al. 1983). The possibility should therefore be considered that glycyl-glutamine may act as a low-molecular weight inhibitory messenger regulating neurotransmission.

β-endorphin and opiate receptors

We investigated the opiate-like binding properties of β-endorphin(1–31) to synaptosomes from rat cortex using carrier-free radio-labelled β-endorphin with 125I-iodine attached specifically to the tyrosine residue at position 27 of the human sequence. Using this system, we were able to demonstrate the existence of binding sites that had a higher affinity for the 31-residue peptide than for any of its naturally occurring relatives,
including β-endorphin(1–27) (the C-fragment), β-endorphin(1–26) (des-histidine C'-fragment) and β-endorphin(1–17) (γ-endorphin) as well as enkephalin and a range of classical ligands (Toogood et al. 1986).

It was observed that relative to β-endorphin, the binding affinities of the shorter forms of β-endorphin were considerably higher than the corresponding analgesic potencies. This could suggest that the β-endorphin receptors in the brain subserve functions other than or in addition to analgesia.

Our results, taken together with the studies of Goldstein and his colleague on dynorphin binding sites (Chavkin & Goldstein 1981), suggested that opiate receptors interact with complementary opioid peptides, β-endorphin acting preferentially at a ‘β-endorphin receptor’ (possibly the ε receptor present in rat vas deferens (Hammonds et al. 1984) or the μ receptor in guinea pig ileum (Paterson et al. 1983)), dynorphin at a ‘dynorphin receptor’ (the κ receptor (Chavkin & Goldstein 1981)) and enkephalin at an ‘enkephalin receptor’ (the δ receptor present in mouse vas deferens (Wuster et al. 1979)). The hypothesis proposed that each functional opioid peptide acts at a different receptor, permitting a diversity of response.

β-endorphin in pituitary: tissue-specific processing

In rat pituitary, we showed that β-endorphin was present specifically in the corticotrophs (Zakarian & Smyth 1979, 1982a) (Fig. 5). This was in line with the important discovery made in Herbert’s laboratory in Portland, Oregon, that lipotropin and ACTH are formed from a common precursor (Mains et al. 1977, Roberts & Herbert 1977). β-endorphin immunoreactivity was present in all the cells of the pars intermedia but none in the posterior pituitary. We had by this time identified six immunoreactive forms of β-endorphin (Smyth et al. 1978, 1981a,b, Smyth 1984), which were generated by C-terminal proteolysis or N-terminal acetylation (Smyth et al. 1979), and only β-endorphin(1–31) possessed potent analgesic activity. This showed that both the N- and C-terminal sequences of β-endorphin are essential for its potent analgesic activity (Geisow et al. 1977, Deakin et al. 1980).

The predominant form of β-endorphin in rat anterior pituitary was β-endorphin(1–31), but the principal forms in the pars intermedia were acetylated at their N-terminus and/or truncated at their C-terminus (Smyth & Zakarian 1980, Smyth 1981, Smyth et al. 1981a,b, Zakarian & Smyth 1982a) (Fig. 6). The processing patterns were demonstrated by extracting the peptides from the tissues, resolving them by gel exclusion and ion-exchange chromatography and determining their concentrations by radioimmunoassay.

We found that alternative processing mechanisms exist for fragmentation of pro-opiomelanocortin, the multifunctional prohormone of β-endorphin, mechanisms that generate different biological activities in different tissues. Of interest was the finding that activation of the immune system by tissue transplantation in the rat led to significant changes in the processing of β-endorphin in its pituitary (Zakarian et al. 1989). Taken together with the results of experiments conducted in Xenopus laevis (Maruthainar et al. 1992), it is clear that the processing of pro-opiomelanocortin is dynamic: it responds to physiological signals.

It is of interest that, depending on the species, ACTH in the anterior pituitary is sometimes accompanied by variable amounts of β-endorphin(1–31), whereas in the pars intermedia, α-MSH is accompanied by inactive forms.

Figure 5
Localisation of peptides related to β-endorphin (A) in the anterior pituitary and (B) in the pars intermedia of the rat by immunofluorescence scanning. Serial sections of perfused and fixed brain from Lewis rats were stained with the antibody to β-endorphin. Reproduced, with permission, from Zakarian S & Smyth DG, 1982, Biochemical Journal, 202, 561–571. Copyright the Biochemical Society.
of β-endorphin. Acetylation at the N-terminus of α-MSH activates this melanotropic hormone but acetylation at the N-terminus of β-endorphin leads to its inactivation (Smyth et al. 1979). Consequently, the ‘balance’ of the two activities is altered and possibly regulated in vivo by this processing reaction.

The presence of a cascade of peptides originating from a common precursor presented us with a physiological system that could be investigated to advantage. An interesting question was: do the different peptides released from their precursors remain in the same compartment in the secretory granule where the processing reactions occur or do the different processing reactions take place independently in a regimented manner? Evidence was obtained by extracting lipotropin and peptides related to β-endorphin from the anterior pituitary and pars intermedia of the pituitary and then resolving them by gel exclusion and cation exchange chromatography (Smyth et al. 1988). It was envisaged that possible heterogeneity in the structure of lipotropin would be revealed by identifying its C-terminal fragment released by trypsin, cleavage being restricted to the COOH group of arginine at position 60 of the lipotropin sequence, achieved by protecting the ε-NH₂ groups of lysine reversibly by citraconylation. Overall, the strategy was to release β-endorphin(1–31) from lipotropin with an intact C-terminal region, but β-endorphin(1–27) and β-endorphin(1–26) from lipotropin truncated at the C-terminus.

The lipotropin obtained from both the anterior pituitary and the pars intermedia was found to give rise to the same C-terminal peptide: it contained the full 31-residue sequence of β-endorphin. None of the 26- and 27-residue forms was generated. In marked contrast, peptides with a similar size to β-endorphin isolated directly from the pars intermedia exhibited a high degree of C-terminal proteolysis: they were present principally as the 26- and 27-residue forms.

The experiment revealed that lipotropin differs from β-endorphin in that it occurs exclusively in a form that contains the full C-terminal sequence. It can be concluded that during biosynthesis, lipotropin gives rise to β-endorphin before proteolysis takes place at its C-terminus. The processing reactions that convert lipotropin to β-endorphin(1–31) and β-endorphin(1–31) to β-endorphin(1–27) are thus ordered and not competitive, that is, they do not take place simultaneously. Furthermore, it is implicit that the paired basic residue enzyme that releases β-endorphin(1–31) at a Lys–Arg sequence does not act at the Lys–Lys site necessary for the release of β-endorphin(1–27).

In summary, the investigation demonstrated that the prohormone of β-endorphin undergoes proteolytic processing in distinct stages that occur in a time-ordered sequence, each step going to completion before the next commences:

\[
POMC \rightarrow \text{lipotropin}(1–91) \rightarrow \text{lipotropin}(61–91) \rightarrow \text{lipotropin}(61–87) \rightarrow \text{lipotropin}(61–86)
\]

![Figure 6](image-url)
The results suggested an additional point of interest that the processing enzymes must be sequestered in different compartments. If they were present in the same compartment, the enzyme that acts at the Lys–Lys sequence in β-endorphin would be expected to act at this same sequence in lipotropin. The experiments showed that the cleavage took place exclusively on the β-endorphin and not at all on the lipotropin. In conclusion, the experimental results indicated that processing enzymes with different specificity appear to be located in different cells or secretory granules.

**Processing of β-endorphin and melanotropin: sensitivity to physiological signals**

Evidence that hormone activity is regulated physiologically at the level of prohormone processing was obtained by studying the acetylation of β-endorphin and α-MSH in Xenopus laevis (Maruthainar et al. 1992). Against a dark background, frogs use darkening of the skin to conceal themselves from predators by blending with their surroundings. We found that the principal form of MSH in the pars intermedia of the dark-adapted frogs was the acetylated peptide, which is the potent form of the hormone. In contrast, the main form of MSH in the pituitary of frogs maintained in a daylight environment was the ‘non-acetylated’ peptide with a basic N-terminal NH$_2$ group, which possesses only weak melanotropic activity (Fig. 7). Thus, the melanotropic activity of α-MSH in background adaptation is governed by specific processing of the ACTH–endorphin prohormone, probably by the enzyme that catalyses the acetylation reaction.

It is interesting that the processing changes took place over the course of several days. The responses were certainly not instantaneous, which indicates that colour adaptation is likely to involve relatively slow biosynthetic mechanisms. We also found that the processing of β-endorphin changed in response to change in the environment, with respect not only to acetylation but also proteolysis. The acetyl form of β-endorphin(1–8) proved to be the principal peptide on the dark background and non-acetylated β-endorphin(1–31) (the potent form) against the light background.

**β-endorphin and morphine: spatial structure**

Morphine, an alkaloid, exhibits similar biological activity to β-endorphin. We constructed models of these molecules, and it was interesting to see that morphine which has a rigid molecular structure imposed by fused rings was very similar to the N-terminal region of β-endorphin when the peptide chain was folded in the β-turn predicted from its sequence (Bradbury et al. 1976d). There was an even closer similarity with benzyloripavine, a potent synthetic derivative of morphine (Fig. 8). This implied that the opiate activities of β-endorphin and morphine depend on the shape of the molecule rather than its chemistry.

**β-endorphin: message and address regions**

The analgesic potency of β-endorphin(1–27), which lacks the four C-terminal residues of β-endorphin(1–31), is 2 orders of magnitude less than the 31-residue peptide, but the duration of analgesic activity is undiminished (Feldberg & Smyth 1977). The four C-terminal residues of β-endorphin are thus important for the potency but not for the duration of analgesia. It has been suggested that the intact C-terminal region of β-endorphin offers a secondary binding site for analgesic receptors, but no inhibitory effects were observed on investigation of the N-acetyl derivative of β-endorphin(1–31), which contains the intact C-terminal sequence. In addition, we found that the binding of the 27-residue peptide to brain receptors, like that of β-endorphin(1–31), was inhibited by sodium and augmented by magnesium, which indicates that β-endorphin(1–27) and β-endorphin(1–31) act as agonists. It is possible that the contribution of the C-terminal region of β-endorphin to the production of analgesia is related to an interaction between the N- and C-terminal regions of the 31-residue peptide within the environment of a complementary receptor, an interaction that influences the conformation of the N-terminal Tyr–Gly–Gly–Phe.

The finding that the peptide chain of β-endorphin that extends beyond the N-terminal pentapeptide has a profound influence on analgesic activity led us to propose a hypothesis that opioid peptides comprise an active site or ‘message region’, the N-terminal tetrapeptide, linked to an ‘address sequence’ which is formed from the continuing chain of amino acids (Smyth 1980). This concept of message and address was proposed by Schwyzer when he applied it to ACTH (Schwyzer 1977). The message region of β-endorphin appears to provide the binding affinity for opiate receptor, whereas the address confers specificity and potency. This would explain the observation that some opioid peptides have exceptionally high potency in certain opiate assays but far less in others. Dynorphin, for example, is far
more potent than leucine enkephalin in the guinea pig ileum assay, but it does not have outstanding analgesic properties. The message and address hypothesis may also rationalise the finding of \(\kappa\)-specificity in one group of opioid receptors, \(\delta\)-specificity in another and \(\mu\)-specificity in a third, because the peptides that act preferentially at these receptors possess the same four residues at their N-terminus but have different address sequences. Similarly, it appears that the shorter address sequences of \(\beta\)-endorphin(1–27), \(\gamma\)-endorphin (\(\beta\)-endorphin(1–17)) and \(\alpha\)-endorphin (\(\beta\)-endorphin(1–16)) compared with \(\beta\)-endorphin(1–31) may account for their relative lack of analgesic properties.

Methionine enkephalin is biosynthesised from a precursor that contains several copies of the pentapeptide and one copy of Leu-enkephalin, each copy separated by a different sequence of amino acids (Comb et al. 1982). It has been shown that in some species, C-terminally extended forms of enkephalin occur as major peptides in the medulla of the adrenal gland, where they are present in sufficient quantity for identification (Kilpatrick et al. 1981). Interestingly, it was reported that they exhibit higher potency than enkephalin in specific opiate assays. Because of their potent activities, these peptides seem likely to exist in their own right and are unlikely to be merely transient intermediates formed en route to an enkephalin. These findings point to the existence of a spectrum of opiate active peptides that have the same message region but different address sequences and possess different properties in terms of specificity and potency. The concept of a range of opioid peptides derived from a single prohormone, each with its preferred receptor, is credible and it has an aesthetic attraction. It may apply to the biologically active peptides derived from other gene duplicated prohormones.

**\(\beta\)-endorphin and enkephalin: neuromodulator and neurotransmitter**

Peptides in the brain are generally considered to act as neurotransmitters, whereas peptides in the periphery act as hormones. Our experience with \(\beta\)-endorphin and enkephalin indicates that similar principles govern their actions at the two locations.

In the brain, small peptides such as enkephalin resemble classical transmitters such as acetylcholine. They act over short distances across synaptic gaps, have relatively low affinity for receptors (rapid on/off rates) and are broken down rapidly, which explains their

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**Figure 7**

Resolution by ion-exchange chromatography of acetyl \(\alpha\)-MSH and des-acetyl \(\alpha\)-MSH extracted from the pars intermedia of *Xenopus laevis* adapted to black or white backgrounds. The peptides purified by gel exclusion on Sephadex G-50 and ion-exchange chromatography on SP Sephadex C-25 were located by RIA with MSH antibody after (A) 2 weeks adaptation to a daylight background and (B and C) after 2 and 4 weeks adaptation to a black background. Reproduced, with permission, from Maruthainar et al. (1992).
short-lasting evanescent effects. Larger peptides in the brain such as β-endorphin have higher receptor affinities, are much more stable and produce long-lasting effects, consistent with their functioning as neuromodulators that express their functions at more distant targets. In the periphery, large peptides being relatively stable are transported from the pituitary via the circulation to perform their function as hormones, but small peptides may behave in the periphery as transmitters do in the brain: they can act locally as paracrine agents, transmitting signals that modulate hormone activity.

In respect of their activities, the modes of action of both small and large peptides in the brain can be seen to have counterparts in the periphery. Even so, β-endorphin and enkephalin appear to represent different classes of neuroactive peptide, that is to say, ‘neuromodulator’ and ‘neurotransmitter’, each with characteristically distinct properties and distinctive functional roles.

**Opioid peptides: on the identification of structure**

The early pioneers had the foresight to anticipate that the determination of structure would prove to be of outstanding importance in the future. In the event, identification of the structures of enkephalin and β-endorphin laid the foundations for a new era, advancing our knowledge and understanding of chemical transmission in the central nervous system.

In a different field of investigation, Li’s identification of the amino acid sequence of lipotropin (Li et al. 1965) and later the characterisation of γ-lipotropin (Chretien & Li 1967) served as a signpost pointing to the discovery of β-endorphin. It was knowledge of the lipotropin sequence and our identification of β-endorphin in the pituitary that led the way to demonstration of the potent opiate activity of this interesting peptide and revealed its profound significance in brain function.

The identification of enkephalin and β-endorphin offers examples of different approaches used in the isolation of biologically active peptides. In the competitive race for identifying peptides with opiate activity, the possibility that the brain might contain an endogenous form of morphine had been discussed regularly by Goldstein, Hughes, Kosterlitz, Simons and Snyder at Meetings of the International Narcotic Research Conference. The breakthrough came with the identification of enkephalin and β-endorphin. In the case of enkephalin, the activity of the predicted opiate was anticipated, then the structure followed. With β-endorphin, the structure came first, the activity later. Happily all roads lead to Rome!

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