

Zeitschrift:	Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche
Herausgeber:	Schweizerische Akademie der Medizinischen Wissenschaften
Band:	- (1981-1982)
Artikel:	The lipotrophins and endorphins
Autor:	Ratter, Sally J. / Rees, Lesley H.
DOI:	https://doi.org/10.5169/seals-308268

Nutzungsbedingungen

Die ETH-Bibliothek ist die Anbieterin der digitalisierten Zeitschriften auf E-Periodica. Sie besitzt keine Urheberrechte an den Zeitschriften und ist nicht verantwortlich für deren Inhalte. Die Rechte liegen in der Regel bei den Herausgebern beziehungsweise den externen Rechteinhabern. Das Veröffentlichen von Bildern in Print- und Online-Publikationen sowie auf Social Media-Kanälen oder Webseiten ist nur mit vorheriger Genehmigung der Rechteinhaber erlaubt. [Mehr erfahren](#)

Conditions d'utilisation

L'ETH Library est le fournisseur des revues numérisées. Elle ne détient aucun droit d'auteur sur les revues et n'est pas responsable de leur contenu. En règle générale, les droits sont détenus par les éditeurs ou les détenteurs de droits externes. La reproduction d'images dans des publications imprimées ou en ligne ainsi que sur des canaux de médias sociaux ou des sites web n'est autorisée qu'avec l'accord préalable des détenteurs des droits. [En savoir plus](#)

Terms of use

The ETH Library is the provider of the digitised journals. It does not own any copyrights to the journals and is not responsible for their content. The rights usually lie with the publishers or the external rights holders. Publishing images in print and online publications, as well as on social media channels or websites, is only permitted with the prior consent of the rights holders. [Find out more](#)

Download PDF: 21.01.2026

ETH-Bibliothek Zürich, E-Periodica, <https://www.e-periodica.ch>

Department of Chemical Endocrinology, St. Bartholomew's Hospital, London, EC1A 7BE. U.K.

THE LIPOTROPHINS AND ENDORPHINS

SALLY J. RATTER and LESLEY H. REES

Isolation

BLPH

β -lipotrophin (BLPH) - so named because of its fat mobilizing effect - was first isolated from the sheep pituitary gland in 1964 by Li and Co-workers (Li 1964). Structural analysis of the ovine β -LPH revealed a single chain polypeptide of 91 amino acid residues (Li et al. 1965; Graf and Li 1973). Porcine and human pituitary LPH were later isolated and sequenced (Cseh et al. 1968; Graf et al. 1971; Li and Chung 1976). A second naturally occurring, but smaller lipotrophin was also described, this being the N-terminal 58 residues of BLPH and termed γ LPH (Chretien and Li 1967).

Close examination of the β -LPH amino acid sequence revealed some interesting features. There was considerable inter-species variation in the residues of the N-terminal portion of the peptide suggesting that this region of the peptide may not have specific biological action. Residues 41-58, however, are identical to the sequence of β melanocyte stimulating hormone (BMSH). BMSH had been previously isolated from mammalian pituitaries and shown to have pigment dispersing biological activity in lower vertebrates (Dixon 1960) and it was suggested by Chretien and colleagues (1967) that BMSH was synthesised from BLPH with γ LPH acting as an intermediate. However, in the human, this has been shown not to be the case, the BMSH being an artefact formed by the enzymatic breakdown of the LPH molecule during extraction procedures at pH values which did not abolish enzymatic activity. Thus the radioimmuno-assays for human BMSH were in fact measuring other peptides, notably β -LPH in pituitary tissue and β -LPH and γ -LPH in the plasma (Bloomfield et al. 1974; Bachelot et al. 1977). Apart from its relationship with β -MSH and melanocyte dispersion no apparent specific biological function could be ascribed to β -LPH. The weak lipolytic activity first described is non-specific since it is a weak property of all pituitary hormones, separate from their main biological actions. Thus for over a decade the function of β -LPH remained unclear.

Endogenous Opiates

The powerful analgesic property of morphine, an alkaloid isolated from the opium poppy, has long been known – its name being taken from Morpheus the Greek god of dreams. However, its strongly addictive properties limited its clinical usefulness which led to attempts in the early 1970's to synthesise various analogues which might separate the two activities. Minor changes in the structure of these analogues were shown to produce potent agonists or dnt-agonists and thus it became apparent that specific receptors were involved in the action of these receptors (Simon and Hiller 1973; Pert and Snyder 1973). These assays permitted the mapping of the opiate receptors within the brain. They were shown to be present only in vertebrate brains and to be mainly concentrated in the mesolimbic system (Kuhar et al. 1974). The receptors were also shown to exist in 2 conformations which had differing affinities for agonists and antagonists and for sodium ions (Simon and Hiller 1973; Pert and Snyder 1974). It seemed highly unlikely that such specific receptors would be developed in the vertebrate brain for a chemical substance from a poppy and so the search for the natural endogenous opiate began. To aid the search, radio-receptor and bioassays were developed and used. The bioassays measured the opiate inhibition of electrically induced contractions of guinea pig ileum or mouse vas deferens (Kosterlitz et al. 1970; Hughes et al. 1975a). The displacement of bound radiolabelled opiate from brain homogenate by unknown opiates was first described as an opiate assay by Terenius and Wahlstrom (1975).

The first endogenous opiates to be identified were isolated from pig brains by a combination of methods including the bioassays, gel filtration, ion exchange and thin-layers chromatography and amino acid analysis (Hughes et al. 1975b). Hughes described two related pentapeptides – methionine enkephalin (Try-Gly-Gly-Phe-Met) and leucine enkephalin (Try-Gly-Gly-Phe-Leu) – and showed the synthetic peptides to have the same activity as the naturally occurring ones. Simantov and Snyder, using beef brains, confirmed these results and described the greater abundance of leucine enkephalin over methionine enkephalin in the beef brain (Simantov and Snyder 1976).

Meanwhile, in 1975, Goldstein's group found opiate-like material in crude preparations of ACTH although synthetic ACTH or α MSH lacked any opiate activity (Cox et al. 1975). This prompted several groups to look more closely at the pituitary for potential endogenous opiates. The crude preparation of Cox was further purified and a peptide with opiate activity was isolated with a molecular weight of some 1750 daltons (Teschemacher et al. 1975). Bradbury and colleagues (1975) described a pituitary peptide of 31 amino acids which was opiate bioactive. They recognised the sequence of this peptide as being identical to the C-terminal 61-91 portion of the β -LPH molecule. This peptide was termed "C-fragment" and could be

prepared from β -LPH by trypsin digestion. It was more potent in the opiate assays than either met-enkephalin or morphine and it was noted to contain the pentapeptide sequence of met-enkephalin which lead to the postulation that it could act as a precursor for this smaller peptide. At the same time Li and Chung were extracting camel pituitaries and they also described the isolation and sequencing of an untriacontapeptide corresponding to β -LPH 61-91. After a suggestion from Eric Simon, they called this peptide β -endorphin - endogenous morphine (Li and Chung 1976). Human β -endorphin, isolated from human pituitary glands, was also described by Li (Li et al. 1976) and reported as having significant opiate activity. Then two more fragments of the β -LPH molecule, both with opiate activity, were isolated from porcine hypothalamic - neurohypophyseal extracts by Ling (Ling et al. 1976). These were termed α -endorphin (β -LPH 61-76) and γ -endorphin (β -LPH)61-77. Thus, an ever increasing number of endogenous opiates were being isolated and characterised from the brains, nervous tissue and pituitaries of vertebrate animals. Interest then became focused on the development of specific assays for these different peptides in order to elucidate their physiological role.

Development of assays

β -LPH and β -endorphin

β -LPH, having no known specific bioactivity, cannot be measured by bioassay and since β -endorphin shares its opiate bioactivity with several other peptides it too cannot be measured specifically by bioassay. Thus, radioimmunoassay becomes the method of choice. However the development of specific and sufficiently sensitive radioimmunoassays for β LPH and β -endorphin is highly problematical because of the shared amino acid sequences between them and a family of related peptides.

Chromatography showed that the radioimmunoassays originally developed for human BMSH (Abe et al. 1967; Donald and Toth 1973; Gilkes et al. 1975) were measuring β -LPH and γ LPH or BMSH as a breakdown artefact of the assay system since it was confirmed that BMSH per se does not exist in the human except possibly in ectopic tumours (McLoughlin 1980). There have been several β -LPH assays reported (Krieger et al. 1977; Wiedemann et al. 1977; Jeffcoate et al. 1978a). These employ an antiserum directed towards the N-terminal β -LPH sequence and thus will not cross react with β -endorphin. When used as plasma assays most of these methods require extraction of the peptide onto porous glass to overcome non-specific interference of the plasma and to also increase sensitivity. The methods described for measuring β -endorphin, use antisera directed to the C-terminal sequence which in most

cases will cross react with β -LPH though not the other endorphins or enkephalin (Guillemin et al. 1977; Jeffcoate et al. 1978b; Akil et al. 1979; Wardlaw and Frantz 1979). Again, several of these methods require peptide extraction when measuring plasma samples, thus introducing the possibility of artefactual breakdown of the large peptides.

Thus, to completely identify the actual peptides β -LPH or β -endorphin and measure them specifically, a combination of radioimmunoassay with gel chromatography or affinity chromatography is required. Assay conditions which do not permit artefactual generation of the smaller peptides from the larger is also important. Several groups of workers have published methods which have achieved this, measuring β -endorphin in human plasma, CSF and tissue (Höllt et al. 1979; Wardlaw and Frantz 1979; McLoughlin et al. 1980; Yamaguchi et al. 1980). Although these combined methodology will measure the specific peptides they are very lengthy to perform and though evaluating the immunoactivity of the peptides this may not correlate with the bioactivity (Smyth 1979).

Enkephalin

The measurement of the enkephalins by opiate bioassay or receptor assay meets the same problems of non-specificity as for β -endorphin and so again radioimmunoassay appears to be the most suitable method. However, yet again, the similar residue sequences of met-leu-enkephalin and of any putative precursors resulted in many radioimmunoassays being non-specific (Simantov et al. 1977; Sullivan et al. 1977; Wesche et al. 1977; Miller et al. 1978). However, a highly specific assay for met-enkephalin was developed by Clement-Jones (1980) which does not cross react with leu-enkephalin or β -endorphin. The assay was developed to measure the methionine sulphoxide analogue of met-enkephalin, all the samples being oxidised with hydrogen peroxide before assay and the antisera raised to ⁵methionine sulphoxide coupled at its N-terminus to thyroglobulin. This antibody does not cross react with leu-enkephalin or any C-terminally extended met-enkephalin peptides. The assay system for plasma employs extraction onto ODS silica, thus removing non-specific interference of plasma proteins whilst permitting concentration of the samples and is carried out at pH 1.5, thus minimising proteolytic activity. An assay for leu-enkephalin has also been reported using cyanogen bromide to remove the met-enkephalin, but the full cross-reactivity studies have not been performed (Ryder and Eng 1981).

Precursors

β -LPH/ β -endorphin

The concept of a common precursor for ACTH and LPH was confirmed using the mouse anterior pituitary tumour cell line At T20/16vD. Mains and Eipper, in a series of experiments first characterised the different molecular weight forms of ACTH and LPH (Eipper and Mains 1975; Mains et al. 1977). Then, using radioactive amino acids, they followed their incorporation into a 31,000 molecular weight peptide containing both ACTH and LPH immunoreactivity. The use of pulse chase studies then demonstrated the conversion of the 31K peptide to 1-39 ACTH and 1-91 LPH (Eipper and Mains 1978). Roberts and Herbert (1977) meanwhile isolated mRNA and membrane bound polysomes from cultures of the AtT20 mouse cells and produced a gene product containing both ACTH and LPH activity within a 28K molecular weight peptide.

In 1979 Nakanishi published the full sequence for the cloned cDNA bovine ACTH/LPH precursor, now known as pro-opiocortin (Nakanishi et al. 1979). This peptide consists of an N-terminal 16K molecular weight portion containing another MSH sequence (γ MSH) joined at its C-terminus to ACTH which in turn is linked to the LPH (Fig. 1). Processing of the pro-opiocortin to release the smaller peptide has since been demonstrated, the final products being different when it occurs in the corticotrophs of the anterior pituitary as compared to the melanotrophs of the intermediate lobe (Eipper and Mains 1980; Jackson et al. 1981). In the anterior pituitary, the pro-opiocortin is cleaved to release mainly 1-39 ACTH and β -LPH with some γ LPH and β -endorphin. However the cleavage products of the intermediate lobe show further processing to give the smaller peptides α MSH, CLIP (ACTH17-39), γ LPH and β -endorphin with very little ACTH or β -LPH. The control of the release of these peptides also differs between the two lobes, the anterior lobe being under the control of CRF from the hypothalamus whilst the pars intermedia peptide release appears to be dopamine controlled.

Evidence for a similar biosynthetic pathway from pro-opiocortin for β -LPH and β -endorphin in the human has come from several different approaches. Immunohistochemical staining for ACTH and LPH have located them as occurring in the same cells and granules within the pituitary (Phifer et al. 1974; Weber et al. 1978). The development of assays for LPH demonstrated the concomitant release of ACTH and LPH into the circulation (Krieger et al. 1977; Jeffcoate et al. 1978a), whilst the investigation of human pituitary tumour cells *in vitro* has also shown the co-ordinate release of ACTH, LPH and β -endorphin in approximately molar ratio (Gilles et al. 1980) (Fig. 2). More recently the complete amino acid sequence of the human pro-opiocortin has been determined (Seidah et al. 1981).

PRE-PRO OPIOCORTIN & RELATED PEPTIDES

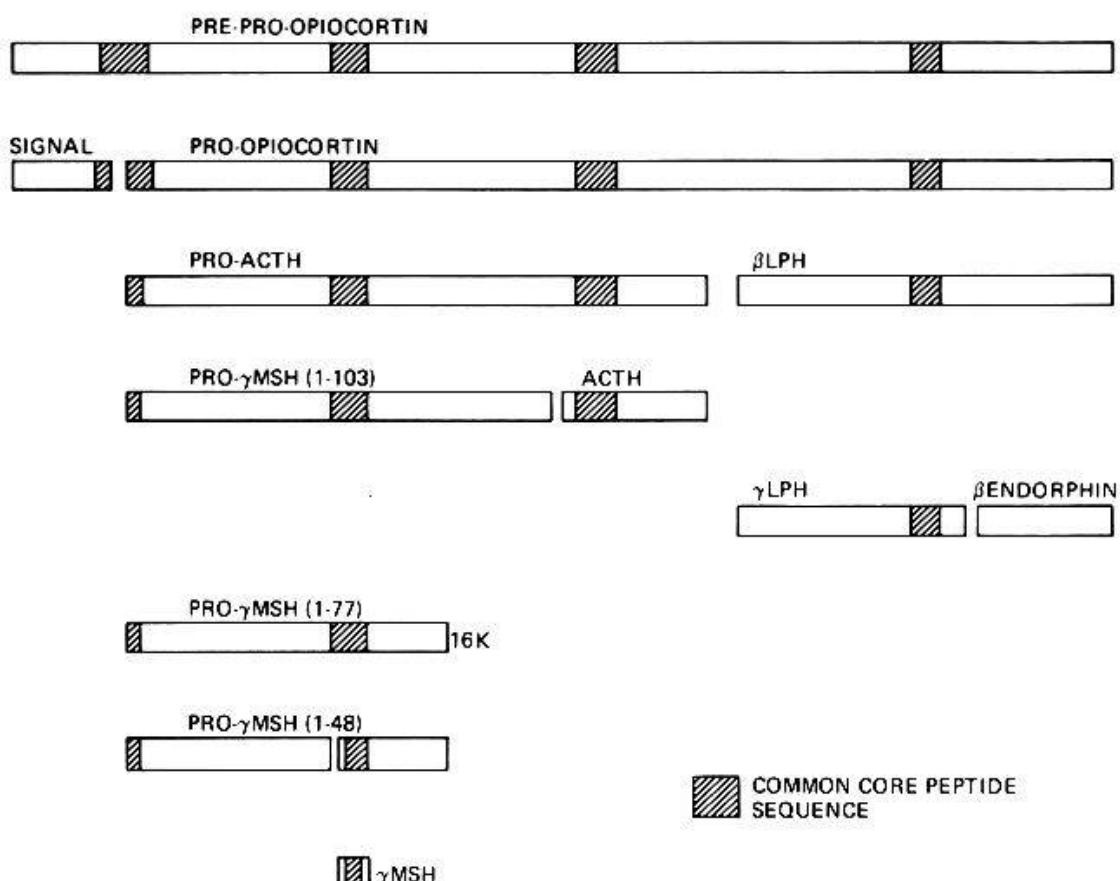


Fig. 1. Schematic representation of the relation of β -LPH, β -endorphin and ACTH to their common precursor pro-opiocortin.

Enkephalins

The appearance of the pentapeptide amino acid sequence of met-enkephalin in β -LPH (β LPH 61-65) led to the belief that β -LPH and β -endorphin acted as a precursor for met-enkephalin. This was supported by the isolation of a brain enzyme which could release met-enkephalin from β -endorphin (Austen et al. 1977). However, the sequence of leu-enkephalin does not occur anywhere in the pro-opiocortin sequence and the pulse-chase experiments of Eipper and Mains failed to show any further processing of β -LPH or β -endorphin to met-enkephalin. It then became apparent that the localisation of β -endorphin and met-enkephalin within the tissues was different. β -endorphin is found chiefly within the pituitary and the hypothalamus whilst met-enkephalin is more widely distributed in the brain and nervous system.

The discovery of considerable amounts of enkephalin-like material within the adrenal medulla (Schultzberg et al. 1978) prompted several groups to look for potential enkephalin precursors

Simultaneous release of ACTH ●—●
 N-LPH ○—○ and pro γ - MSH ○---○ from
 isolated human pituitary tumour cells

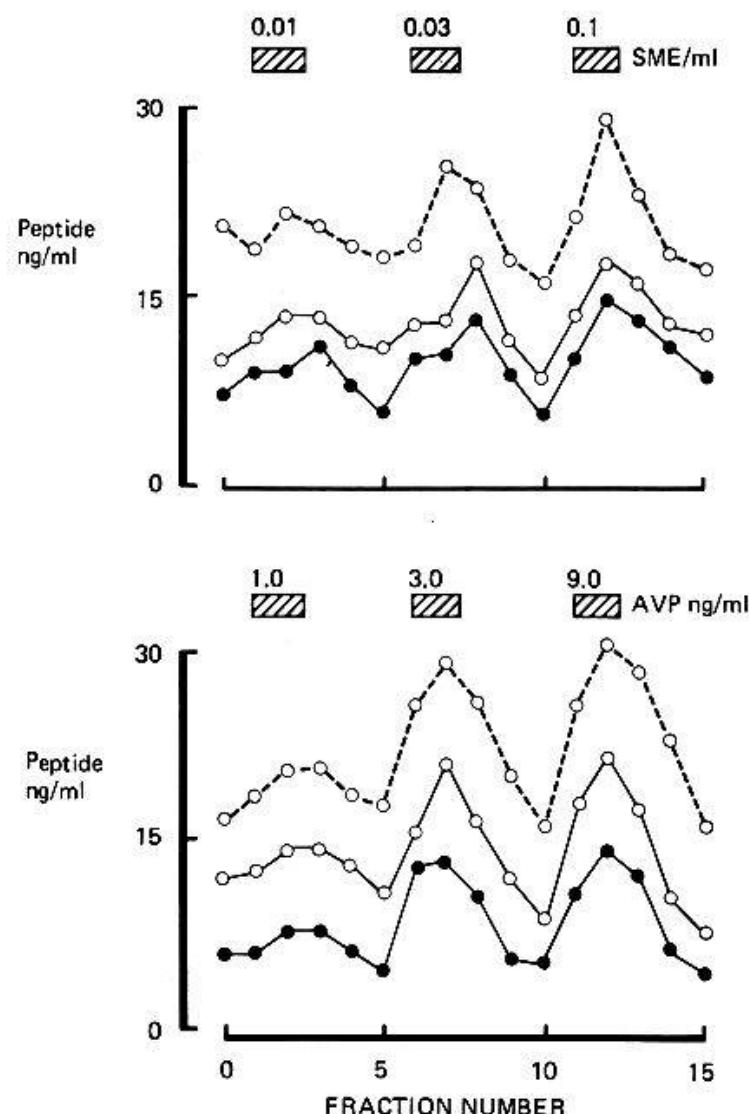


Fig. 2. Peptide release from isolated human pituitary tumour cells in response to: i) upper graph rat stalk median eminence extract (SME). ii) lower graph arginine vasopressin
 ●—● ACTH 0—○ N-LPH 0---○ pro- γ -MSH

within the medullary tissue. A number of different peptides have since been isolated from the adrenal medulla, all containing one or more enkephalin sequences (Yang et al. 1979; Stein et al. 1980; Clement-Jones et al. 1980b). Treatment of these peptides with either trypsin or carboxypeptidases release the pentapeptide enkephalins and opiate bioactivity, many of the larger peptides being opiate inactive. Two putative precursors for leu-enkephalin have also been described, one being isolated from pig hypothalamus and called aneo-endorphin (Kangawa et al. 1979). The second peptide, dynorphin, was isolated from pig pituitaries (Goldstein

et al. 1979). Both of these peptides contained leu-enkephalin as their N-terminal sequence. Confirmation of the potential enkephalin precursor role of these peptides is still required, although some pulse-chase studies have been undertaken (Tan and Yu 1980).

Thus, it has become apparent that β -endorphin and met- and leu-enkephalin, having first been thought to come from a common source, are synthesised by different systems in different locations and may thus play very different physiological roles.

β -LPH, β -endorphin and enkephalin in the circulation

The role of the β -LPH, β -endorphin and the enkephalins in the circulation is uncertain and a search for one has been complicated by the problems of specific assays for the different individual peptides.

There have been various reports on the levels of β -LPH in the plasma in normal and pathological conditions (Krieger et al. 1977; Jeffcoate et al. 1978). These studies have used N-terminal LPH immunoassays, thus measuring β -LPH and γ LPH. They report a good correlation between ACTH and LPH in plasma in normal subjects under basal and stimulated conditions and in patients with disorders of the hypothalamic-pituitary-adrenal axis. In most cases β -LPH and ACTH were related on a 1:1 molar basis. This is now what would be expected with both peptides being released from the common precursor pro-opiocortin.

Confirmation of the presence of β -endorphin in the circulation has been more controversial and problematical due to the possible artefactual generation of β -endorphin from β -LPH and the non-specificity of the C-terminal LPH assays. Problems of sensitivity of the assays may also have confused the issue since Suda and colleagues (1978) could not detect β -endorphin in extracts of normal plasma. The same group also reported the generation of β -endorphin from β -LPH by the use of acetic acid for extraction from the pituitary and the use of silicic acid for extraction from plasma (Liotta et al. 1978 and Suda et al. 1978).

However McLoughlin (1980) using appropriate assay conditions with no prior extraction of plasma and chromatography under acid dissociating conditions, clearly demonstrated β -endorphin in human plasma in both normals and patients with various disorders of the hypothalamic-pituitary-adrenal axis (Fig. 3). Similar results were also given by Wardlaw and Frantz (1979) who also showed that their talc extraction step did not generate β -endorphin. The origin of the β -endorphin remains a little unclear since it is reported as not being detected in the extracts of normal adult human pituitary (Liotta et al. 1978). However Mains and Eipper demonstrated its synthesis from pro-opiocortin in the mouse tumour cell line. Since, it has been shown that pro-opiocortin is not normally released from the pituitary into the circulation (Ratter et al. 1980). It is possible, therefore, that ACTH and β -LPH are

SEPHADEX G50 PLASMA C-TERMINAL βLPH ELUTION PROFILES

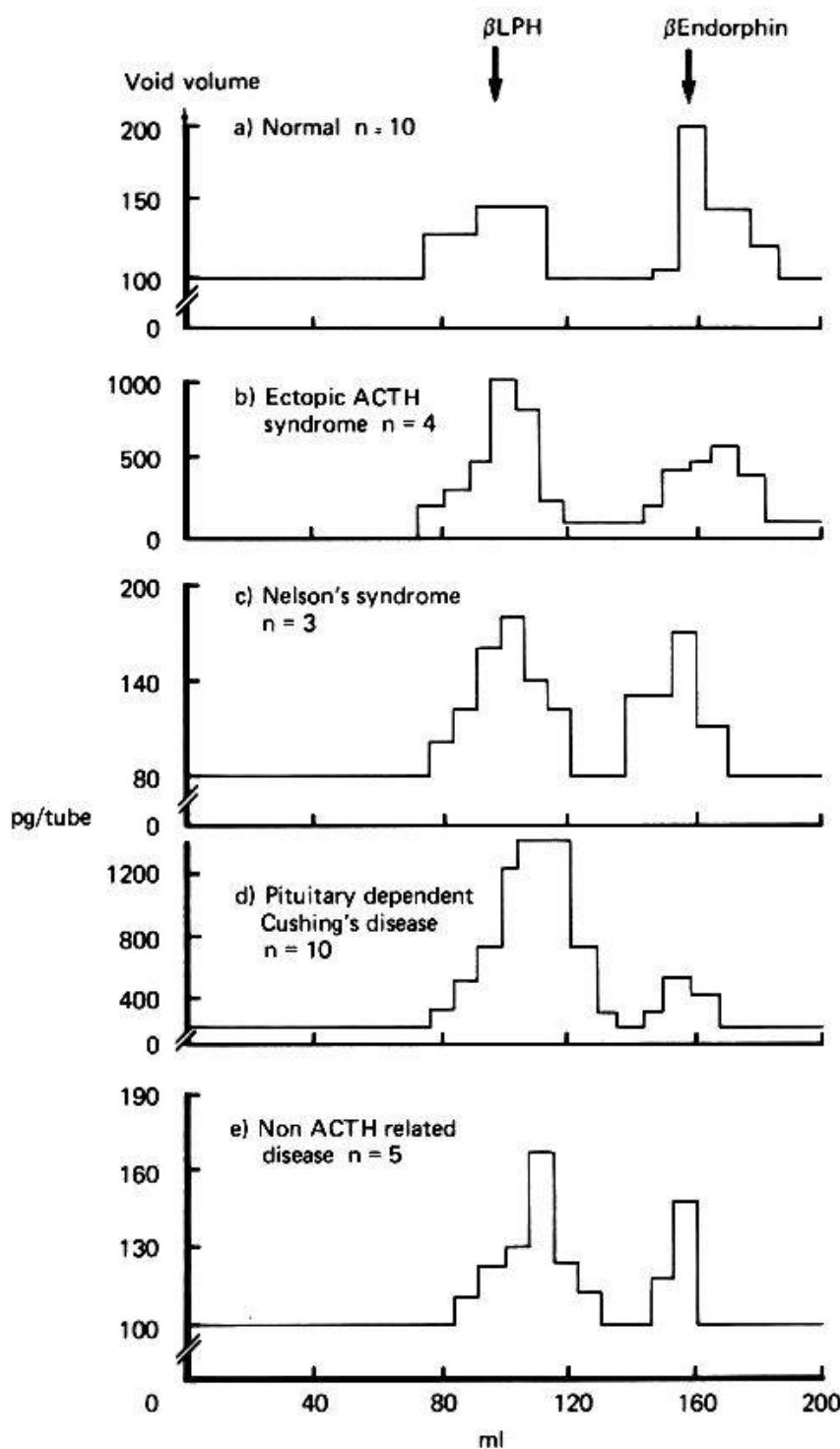


Fig. 3. Chromatographic profiles of C-terminal LPH activity in human plasma. Each profile is of one plasma sample representative of its group taken from normal subjects or patients with various disorders of the hypothalamic-pituitary-adrenal axis.

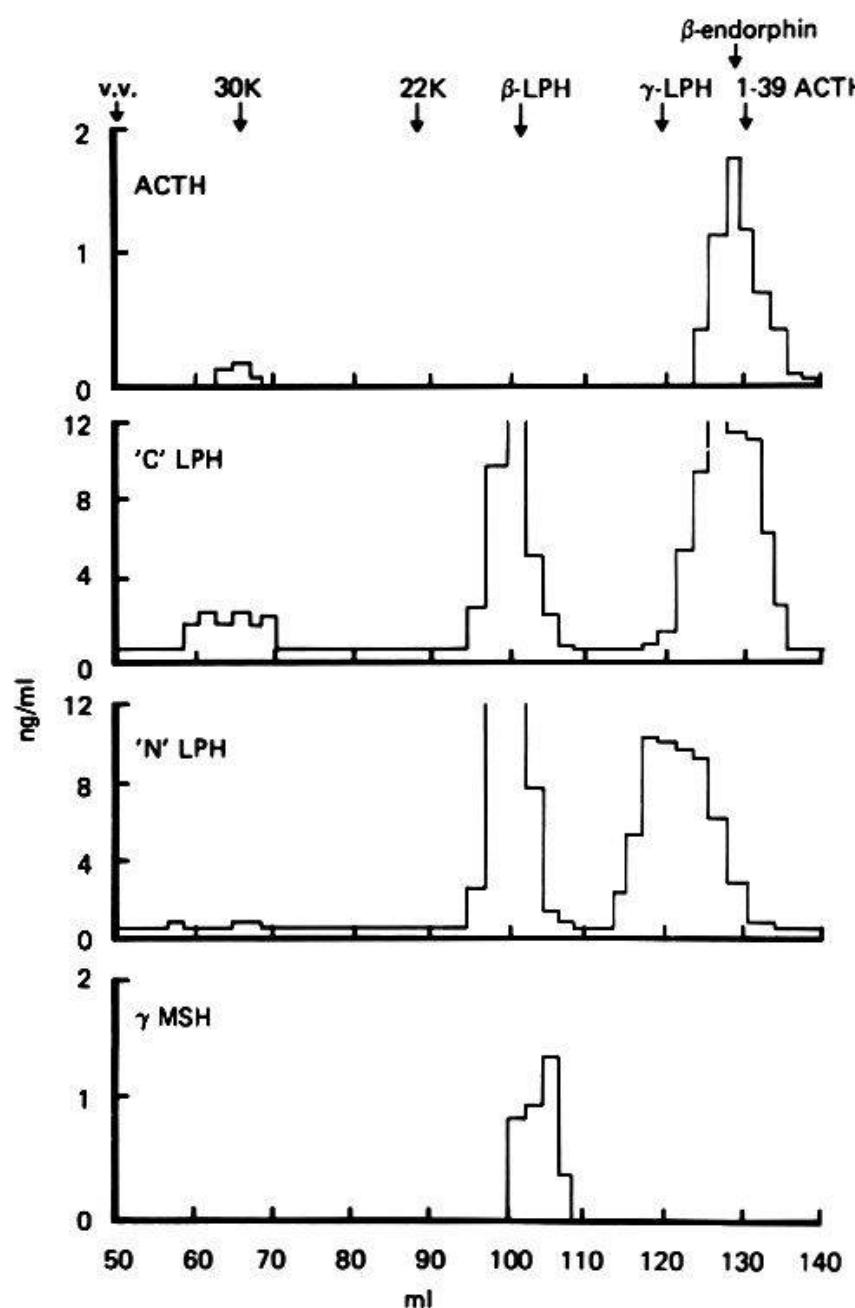


Fig. 4. Chromatographic profiles of ACTH, C-LPH, N-LPH and γ -MSH activity in pituitary tumour cell column effluent chromatographed on Sephadex G-75.

cleaved from their precursor at or just before release from the pituitary cells and that β -endorphin is then processed from the β -LPH at secretion or within the circulation. Human pituitary tumour cells in a dynamic *in vitro* system with conditions which permitted only minimal artefactual breakdown of peptides, clearly demonstrated the release of 1-39 ACTH, β -LPH, γ -LPH and β -endorphin (Gilles et al. 1980) (Fig. 4). Smith and colleagues (1981) have recently carried out a study comparing the plasma levels of ACTH, β -LPH and β -

endorphin and have shown parallel secretion in the variety of stimulatory and inhibitory circumstances which they studied.

The presence of met-enkephalin in human plasma was confirmed by Clement-Jones et al. (1980) with the highly specific assay developed to the methionine sulphoxide previously described. The peptide was concentrated using extraction onto ODS which also remove non-specific plasma protein interference. Levels at 9.0am in normals were shown to range from 14-140pg/ml and chromatography on Biogel P₄ showed a peak eluting in the position of met-enkephalin with 2 minor peaks of the oxidised tetra and pentapeptide. The origin of the met-enkephalin in the plasma is uncertain, although in the brain the precursor for enkephalins has been shown to be distinct from β -endorphin and its precursors. Of course it is possible that some of the met-enkephalin in the circulation could be generated from β -endorphin. However, Clement-Jones could show no increase in met-enkephalin in plasma which contained exogenous or endogenous β -LPH and β -enorphin and was incubated for several hours at room temperature (Clement-Jones et al. 1980) and furthermore Smith and colleagues (1981) reported no relationship between plasma met-enkephalin and plasma β -endorphin. Thus, when dexamethasone administration suppressed the latter to undetectable levels the met-enkephalin was unaltered. Similarly, when β -endorphin levels were raised by hypoglycaemia or in patients with Nelson's syndrome or Addison's disease the met-enkephalin levels were normal. Clement-Jones et al. (1980) demonstrated elevated levels of met-enkephalin in the adrenal vein and with the location of potential enkephalin precursors within the adrenal medulla (Yang et al. 1979, Stein et al. 1980, C. Jones et al. 1980b), it seems possible that some of the circulating met-enkephalin is of adrenal origin. However, against the adrenal as the only source of circulating met-enkephalin is the observation of Smith et al. (1981) that total adrenalectomy did not eliminate met-enkephalin in the circulation, so other sources are probable.

Thus, although we can speculate that the biological role for plasma β -endorphin may relate to stress since it is co-ordinately released with ACTH in response to stress, hard facts concerning the function of both plasma β -endorphin and enkephalin are as yet unknown.

Endorphins and Enkephalins in CSF

Measurement of CSF levels of the natural opiates enables an assessment of their potential roles within the CNS. The radioreceptor binding assay has been used by several groups to study endorphin levels in CSF and their relationship to pain (Almay et al. 1978), psychotic states (Lindstrom et al. 1978) and electroacupuncture (Sjölund et al. 1977). However as mentioned previously these assays do not give information regarding levels of specific

SEPHADEX G75 CSF ELUTION PROFILES

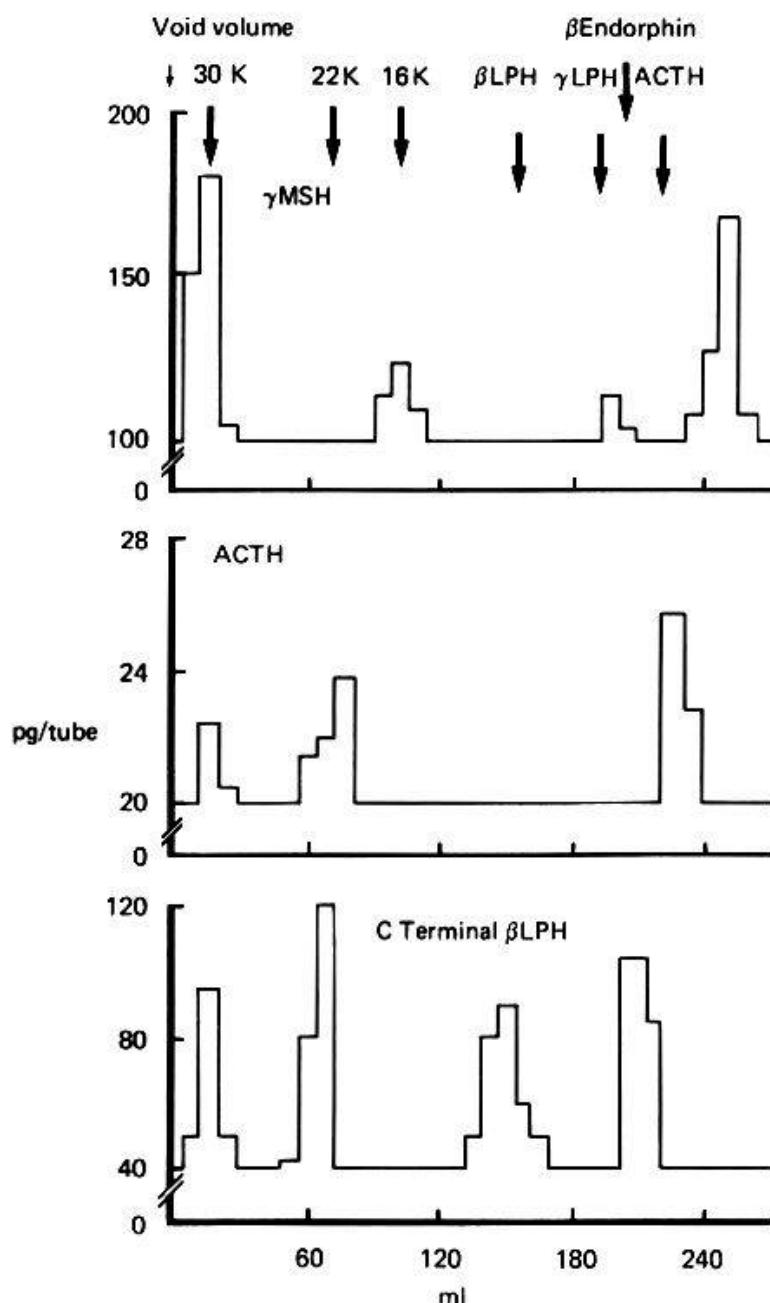


Fig. 5. Chromatographic profiles of pro- γ -MSH, ACTH and C-LPH activity in human CSF chromatographed on Sephadex G-75.

peptides. McLoughlin et al. (1980b) published a characterisation of the β -endorphin related peptides in CSF using a combination of radioimmunoassay and gel chromatography. They demonstrated the presence of β -endorphin as well as β -LPH, γ LPH and 1-39 ACTH in the CSF of patients with a range of non-endocrine disorders and also with ACTH related disease (Fig. 5). Unlike the plasma, the CSF profiles showed significant amounts of larger molecular weight precursors. They postulated that, unlike the release of plasma peptides, the pro-opio-

cortin itself is released into the CSF with subsequent extracellular formation of the smaller peptides. Jeffcoate et al. (1978b) suggested that β -endorphin in CSF might originate from the pituitary by retrograde transport in the hypophyseal portal vessels in the pituitary stalk. However β -endorphin has been measured in the CSF of hypophysectomised subjects and levels in CSF appear to be independent of those in plasma (Jeffcoate et al. 1978b). Thus there may be a separate site of synthesis for the β -endorphin related peptides in CSF and in plasma. Clement-Jones et al. (1980a) used a specific met-enkephalin radioimmunoassay to demonstrate its presence in human CSF in all the subjects investigated including those with pan-hypopituitarism. Levels were lower than reported for plasma and for "enkephalin-like material" in CSF measured by receptor assays (Wahlstrom et al. 1976; Akil et al. 1978a).

Physiological Role for Endorphin and Enkephalins

There is ever growing evidence that the enkephalins play a neuromodulator role in the CNS. They have been located within the CNS by immunohistochemistry, radioimmunoassay and radioreceptor assay in areas where previously the opiate receptors had been found. (Hökfelt et al. 1977; Simantov et al. 1977 and Sar et al. 1978). Miller and Pickel (1980) described the enkephalins as being present in vesicles associated with synapses which have opiate receptors. Met- and leu-enkephalin have both been shown to presynaptically effect - by inhibition - the transmission at sympathetic ganglia (Konishi et al. 1979; Wouters and Van den Bercken 1980). The distribution of β -endorphin, however, is quite separate from the enkephalins, being located in cells in or near the arcuate nucleus. These cells have a wide-spread system of fibres spreading throughout the limbic system, thalamus, midbrain and medulla (Bloom et al. 1978; Watson et al. 1978). A neuromodulator or neurotransmitter role for β -endorphin has, as yet, to be elucidated.

The pain relieving properties of morphine strongly suggest that the natural opiates play a part in pain perception and threshold. Thus, many areas where enkephalins and endorphins are located are also known to be associated with pain perception. The enkephalins are only weakly analgesic when administered intraventricularly and have no effect intravenously (Bellugi et al. 1976). Some synthetic analogues which are more resistant to degradation have much greater analgesic activity intraventricularly. However, intraventricularly administered β -endorphin is a much more potent analgesic with considerably longer lasting effects (Graf et al. 1976; Loh et al. 1976) and also has analgesic properties when given intravenously (Tseng et al. 1976). Naloxone - the opiate antagonist - has been used to investigate the physiologic importance of the natural opiates in pain. If enkephalin and endorphin modulate pain perception and pain threshold then naloxone should increase the former and lower the latter. However, to date

there have been conflicting reports of the effects of naloxone and further studies are required to clarify its action (Goldstein et al. 1976; Buchsbaum et al. 1977; Levine et al. 1978). Some of the observed differences may relate to the different doses of naloxone administered.

Another approach to the investigation of the natural opiates and pain has been to look at the levels of enkephalins and endorphins in patients undergoing acupuncture and electrical treatment for the relief of pain. There have been various reports of increased CSF endorphin levels in subjects being given electrical stimulation for pain relief (Sjölund et al. 1977; Akil et al. 1978b; Clement-Jones et al. 1980d). No equivalent rise was detected in the CSF levels of met-enkephalin (Clement-Jones et al. 1980d). However naloxone was observed to reverse the pain relief given by the treatment (Akil et al. 1978a). Thus, these studies strongly indicate that the pain relief given by such treatment is mediated through the release of endorphins. Acupuncture is also used for the relief of symptoms of withdrawal in heroin addicts. Levels of CSF and plasma β -endorphin are raised in withdrawal whilst CSF met-enkephalin is lowered (Clement-Jones et al. 1979). However during acupuncture the CSF met-enkephalin rose correlating with the degree of symptom relief suggesting the involvement of enkephalin in the analgesia of the acupuncture.

The possible behavioural effects of the natural opiates, especially the endorphins, have also roused considerable interest. In animal experiments, when administered intraventricularly, β -endorphin has been shown to induce such effects as excessive grooming (Gispen et al. 1976) and "wet-dog shakes" (Holaday et al. 1977). Chronic administration of endorphins in rats produced the development of self-administering behaviour and the induction of physical dependence (Belluyi et al. 1977; Tseng et al. 1977). In man, endorphins have been indicated as being involved in various psycho-pathological disorders. Thus, Lindstrom et al. (1978) reported increased levels of "endorphin-like" material in CSF from schizophrenic patients whilst naloxone appears to alleviate some of the psychotic symptoms (Gunne et al. 1977; Lahmann et al. 1979). Veshoeven et al. (1979) described clinical studies using des-tyrosine endorphin (DT γ E) to treat chronic schizophrenics. A reduction in their psychotic symptoms was observed in most of the patients and it was suggested that some types of schizophrenia might be due to a disturbance in the balance of the various endorphins. Endorphins and enkephalins have also been suggested to effect some behavioural modifications seen in patients with neoplasia when there is no evidence for the presence of cerebral metastases.

There have been many reports of the effects of endorphins and enkephalins and their analogues on pituitary hormone release, such that it has been suggested that there may be a specific role for the opiates in pituitary control.

Intravenous administration of enkephalin and its analogues results in rises in plasma prolactin and growth hormone in man (Stubbs et al. 1978; Von Graffenreid et al. 1978) whilst β -endorphin has been shown to induce an increase in prolactin, but not growth hormone (Catlin et al. 1980; Reid et al. 1981). Low doses of naloxone did not influence prolactin or growth hormone although using larger doses, Morley et al. (1980) was able to show an augmented response of prolactin to TRH. No endorphin stimulated prolactin response was seen in stalk sectioned monkeys (Wardlaw et al. 1980), indicating a hypophyseal action rather than direct pituitary and there is now evidence to suggest that the opiate effects are mediated by dopamine suppression (Gudelskey and Porter 1980; Van Loon et al. 1980).

LH and FSH release following administration of naloxone or DAMME - an opiate agonist - has been shown to be suppressed in man by the latter (Stubbs et al. 1978) and stimulated by the former (Morley et al. 1980). Later studies involving LH and FSH release throughout the menstrual cycle demonstrated a varied response of LH to the opiates depending on the stage of the cycle, whilst no effects on FSH were detected (Moult et al. 1981). This led to the suggestion that the opiates alter LH through and LHRH inhibiting effect. These results are consistent with the known reduction of sexual and reproductive function in opiate addicts.

ACTH release and thus cortisol has been reported to be inhibited by DAMME and stimulated by naloxone (Morley et al. 1980; Gaillard et al. 1981). The response of vasopressin release to opiates has not been clarified. Thus, β -endorphin was reported to stimulate vasopressin release in man, though the release was not naloxone reversible (Weitzmann et al. 1977; Firemark and Weitzmann 1979), but Grossman et al. (1980) recorded a suppression of vasopressin by DAMME whilst no effect was found using DAMME by Lightman et al. (1980). Iverson et al. (1980) using electrical stimulation of neurones suggested that there are inhibitory opiate receptors on the terminals of vasopressin fibres.

Intravenous administration of β -endorphin, enkephalin or their analogues have not been shown to be accompanied by any significant changes in heart rate, respiration, blood pressure or liver and renal function (Stubbs et al. 1980; Catlin et al. 1979; Reid et al. 1981). However, subjective symptoms of "tingling", tightness in the neck, epigastric "hunger" and dryness of the mouth were recorded in the same studies.

Endorphins and Enkephalins in the Gastrointestinal Tract

Opiate receptors were demonstrated to be present throughout the gut (Höllt and Wuster 1978) which led, as with the brain, to the search for endogenous opiates in the G.I. tract. Immunocytochemistry demonstrated enkephalin in the myenteric plexus of the intestine, gallbladder

and cystic duct and in the APUD cells of the gastric antral mucosa, duodenal mucosa and pancreas. Opiates have an effect throughout the G.I.T. altering the mobility, muscular control and internal pressures of the gut. They also decrease pancreatic secretion whilst increasing gastric acid secretion. The constipating effect of opiates is well known, but it remains unclear as to whether this is a central, or peripheral action of the opiates. Endorphins and enkephalins may play a role in mediating the psychophysiologic symptoms found in G.I.T. disorders.

Conclusion

There has been a tremendous increase in our knowledge and understanding of the distribution and physiological roles of endorphins and enkephalins. However as we understand more about the different actions of the different peptides we appreciate how much more research is required in the field of endogenous opiates.

- Abe K., Nicholson W., Liddle G.W., Orth D.N., Island D.P. 1967: Radioimmunoassay of β -MSH in human plasma and tissues. *J. Clin. Invest.* 46, 1609-1616.
- Akil H., Richardson D.E., Hughes J., Darchas D. 1978: Enkephalin-like material elevated in ventricular cerebrospinal fluid of pain patients after analgesic focal stimulation. *Science*. 201, 463-465.
- Akil H., Watson S.J., Barchas J.D., Li C.H. 1979: β -endorphin immunoreactivity in rat and human blood: radioimmunoassay, comparative levels and physiological alterations. *Life. Sci.* 24, 1649-1666.
- Almay B.C.L., Johansson F., Von Knorring L., Terenius L., Wahlström A. 1978: Endorphins in chronic pain. Differences in CSF endorphin levels between organic and psychogenic pain syndromes. *Pain*. 5, 153-162.
- Austen B.M., Smyth D.G., Snell C.R. 1977: γ -endorphin, α -endorphin and met-enkephalin are formed extracellularly from lipotrophin C fragment. *Nature*. 269, 619-621.
- Bachelot I., Wolfsen A.R., Odell W.D. 1977: Pituitary and Plasma Lipotrophins: Demonstration of the artefactual Nature of β -MSH. *J. Clin. Endoc. Metab.* 44, 939-946.
- Belluzzi J.D., Grant N., Garsky V., Sarantakis D., Wise C.D., Stein L. 1976: Analgesia induced *in vivo* by central administration of enkephalin in rat. *Nature*. 260, 625-626.
- Belluzzi J.D., Stein L. 1977: Enkephalin may mediate euphoria and drive-reduction reward. *Nature*. 266, 556-558.
- Bloom F.E., Battenberg E., Rossier J., Ling N., Guillemin R. 1978: Neurons containing β -endorphin in rat brain exist separately from those containing enkephalin: Immunohistochemical studies. *Proc. Natl. Acad. Sci. USA*. 75, 1591-1595.
- Bloomfield G.A., Scott A.P., Lowry P.J., Gilkes J.J., Rees L.H. 1974: A reappraisal of human β -MSH. *Nature*. 252, 492-493.
- Bradbury A.F., Smyth D.G., Snell C.R. 1975: In Peptides Structure and Biology (eds. by Meienhofer J.) 609-615. Ann. Arbor. Sci. Inc.
- Buchsbaum M.S., Davis G.C., Bunney W.E. Jr. 1977: Naloxone alters pain perception and somatosensory evoked potentials in normal subjects. *Nature*. 270, 620-622.
- Catlin D.H., Hui K.K., Loh H.H., Li C.H. 1979: β -endorphin: initial clinical studies In: Usdin E., Bunney N., Kline N. (eds.) Endorphins in Mental Health Research. Oxford University Press, New York. p. 535.

- Catlin D.H., Poland R.E., Gorelick D.A., Geines R.H., Hue K.K., Rubin R.T., Li C.H. 1980: Intravenous infusion of β -endorphin increases serum prolactin, but not growth hormone or cortisol, in depressed subjects and withdrawing methadone addicts. *J. Clin. Endoc. Metab.* 50, 1021-1025.
- Chretien M., Li C.H. 1967: Isolation, purification and characterisation of γ -lipotrophic hormone from sheep pituitary glands. *Can. J. Biochem.* 45, 1153-1174.
- Clement-Jones V., Lowry P.J., McLoughlin L., Besser G.M., Rees L.H., Wen H.L. 1979: Acupuncture in heroin addicts: changes in met-enkephalin and β -endorphin in blood and cerebrospinal fluid. *Lancet* ii, 380-383.
- Clement-Jones V., Lowry P.J., Rees L.H., Besser G.M. 1980a: Development of a specific extracted radioimmunoassay for methionine enkephalin in human plasma and cerebrospinal fluid. *J. Endoc.* 86, 231-243.
- Clement-Jones V., Corder R., Lowry P.J. 1980b: Isolation of human met-enkephalin and two groups of putative precursors (2k-pro-met-enkephalin) from an adrenal medullary tumour. *Biochem. Biophys. Res. Commun.* 95, 665-673.
- Clement-Jones V., Lowry P.J., Rees L.H., Besser G.M. 1980c: Met-enkephalin circulates in human plasma. *Nature* 283, 295-297.
- Clement-Jones V., McLoughlin L., Tomlin S., Besser G.M., Rees L.H., Wen H.L. 1980d: Acupuncture for recurrent pain: β -endorphin but no met-enkephalin increases in human cerebrospinal fluid. *Lancet* ii, 946-949.
- Cox B.M., Opheim K.E., Teschemacher H., Goldstein A. 1975: A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties.
- Cseh G., Gráf L. Goth. 1968: Lipotrophic hormone obtained from human pituitary gland. *Febs. Lett.* 2, 42-44.
- Dixon H.B.F. 1960: Chromatographic isolations of pig and human melanocyte stimulating hormones. *Biochem. Biophys. Acta* 37, 38-42.
- Donald R.A., Toth A. 1973: A comparison of the β -melanocyte-stimulating hormone and corticotrophin response to hypoglycaemia. *J. Clin. Endoc. Metab.* 36, 925-930.
- Eipper B.A., Mains R.E. 1975: High molecular weight forms of adreno-corticotrophin in the mouse pituitary tumour line. *Biochem.* 14, 3836-3844.
- Eipper B.A., Mains R.E. 1978: Analysis of the common precursor to corticotrophin and endorphin. *J. Biol. Chem.* 253, 5732-5744.
- Firemark H.M., Weitzman R.E. 1979: Effects of β -endorphin, morphine and naloxone on arginine vasopressin secretion and the electro-enkephalogram. *Neurosci.* 4, 1895-1902.
- Gaillard R.C., Grossman A., Smith R., Rees L.H., Besser G.M. 1981: The effects of a met-enkephalin analogue on ACTH, β -LPH, β -endorphin and met-enkephalin in patients with adrenocortical disease. *Clin. Endoc.* 14, 471-478.
- Gilkes J.J.H., Bloomfield G.A., Scott A.P., Lowry P.J., Ratcliffe J.G., Landon J., Rees L.H. 1975: Development and validation of a radioimmunoassay for peptides related to β -melanocyte-stimulating hormone in human plasma: The Lipotrophins. *J. Clin. Endoc. Metab.* 40, 450-457.
- Gillies G., Ratter S., Grossman A., Gaillard R., Lowry P.J., Besser G.M., Rees L.H. 1980: Secretion of ACTH, LPH and β -endorphin from human pituitary tumours in vitro. *Clin. Endoc.* 13, 197-205.
- Gispen W.H., Wiegant V.M., Bradbury A.F., Hulme E.C., Smythe D.G., Snell C.R., Wied D. de 1976: Induction of excessive grooming in the rat by fragments of lipotrophin. *Nature* 264, 794-795.
- Goldstein A., Pryor G.T., Otis L.S., Larsen F. 1976: On the role of endogenous opioid peptides: failure of naloxone to influence shock escape threshold in the rat. *Life Sci.* 18, 599-604.
- Goldstein A., Tachibana S., Lowney L.I., Hunkapiller M., Hood L. 1979: Dynorphin - (1-13), an extraordinarily potent opioid peptide. *Proc. Natl. Acad. Sci. USA* 76, 6666-6670.

- Graf L., Barat E., Cseh G., Saigo M. 1971: Amino acid sequence of porcine β -lipotrophic hormone. *Biochim. Biophys. Acta.* 229, 276-278.
- Graf L., Li C.H. 1973: Action of plasmin on ovine beta-lipotrophin revision of the carboxyl terminal sequence. *Biochem. Biophys. Res. Commun.* 53, 1304-1309.
- Graf L., Syekely J.I., Ronai A.Z., Dunai-Kovacs Z., Bajusy S. 1976: Comparative study on analgesic effect of met⁵-enkephalin and related lipotropin fragments. *Nature. Lond.* 263, 240-242.
- Grossman A., Besser G.M., Milles J.J., Baylis P.H. 1980: Inhibition of vasopressin release in man by an opiate peptide. *Lancet ii*, 1108-1110.
- Gudelsky G.A., Porter J.C. 1979: Morphine and opioid peptide-induced inhibition of the release of dopamine from tuberoinfundibular neurons. *Life Sci.* 25, 1697.
- Guillemin R., Ling N., Vargo T. 1977: Radioimmunoassays for α -endorphin and β -endorphin. *Biochem. Biophys. Res. Commun.* 17, 361-366.
- Gunne L.M., Lindstrom L., Terenius L. 1977: Naloxone-induced reversal of schizophrenic hallucinations. *J. Neural Trans.* 40, 13-19.
- Hökfelt T., Elde E., Johansson O., Terenius L., Stein L. 1977: The distribution of enkephalin immunoreactive cell bodies in the rat central nervous system. *Neuroscience Lett.* 5, 25-31.
- Holaday J.W., Loh H.H., Li C.H. 1978: Unique behavioural effects of β -endorphin and their relationship to thermoregulators and hypothalamic function. *Life Sci.* 22, 1525-1536.
- Höllt V., Müller O.H., Fahlbusch R. 1979: β -endorphin in human plasma: basal and pathologically elevated levels. *Life Sci.* 25, 37-44.
- Hughes J., Kosterlitz H.W., Leslie F.M. 1975a: Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics.
- Hughes J., Smith T.W., Kosterlitz H.W., Fothergill L.A., Morgan B.A., Morris H.R. 1975b: Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258, 577-579.
- Iversen L.L., Iversen S.D., Bloom F.E. 1980: Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis. *Nature.* 284, 350-351.
- Jeffcoate W.J., Rees L.H., Lowry P.J., Besser G.M. 1978a: A specific radioimmunoassay for human β -lipotropin. *J. Clin. Endoc. Metab.* 47, 160-167.
- Jeffcoate W.J., Rees L.H., McLoughlin L., Ratter S.J., Hope J., Lowry P.J., Besser G.M. 1978b: β -endorphin in human cerebrospinal fluid. *Lancet* 2, 119-121.
- Kangawa K., Matsuo H., Igarashi M. 1978: α -neo-endorphin: a "big" leu-enkephalin with potent opiate activity from porcine hypothalamus. *Biochem. Biophys. Res. Commun.* 86, 153-160.
- Konishi S., Tsunoo A., Otsuka M. 1979: Enkephalins presynaptically inhibit cholinergic transmission in sympathetic ganglia. *Nature.* 282, 515-516.
- Kosterlitz H.W., Lydon R.J., Watt A.J. 1970: The effect of adrenaline, nor-adrenaline and isoprenaline on inhibitory alpha-and-beta-adrenoceptors in the longitudinal muscle of the guinea pig ileum. *Br. J. Pharmacol.* 39, 398-413.
- Krieger D.T., Liotta A., Li C.H. 1977: Human plasma immunoreactive β -lipotropin correlation with basal and stimulated plasma ACTH concentrations. *Life Sci.* 21, 1771-1778.
- Kuhar M.J., Pert C.B., Snyder S.H. 1973: Regional distribution of opiate receptor binding in monkey and human brain. *Nature.* 245, 447-450.
- Lehmann H., Nair N.P.V., Kline N.S. 1979: β -endorphin and naloxone in psychiatric patients: clinical and biological effects. *Am. J. Psychiat.* 136, 762-766.
- Levine J.D., Gordon N.C., Jones R.T., Fields H.L. 1978: The narcotic antagonist naloxone enhances clinical pain. *Nature.* 272, 826-827.
- Li C.H. 1964: Lipotropin, a new active peptide from pituitary glands. *Nature.* 201, 924-925.
- Li C.H., Barnafi L., Chretien M., Chung D. 1965: Isolation and amino-acid sequence of β -LPH from sheep pituitary gland. *Nature.* 208, 1093-1094.
- Li C.H., Chung D. 1976: Primary structure of human β -lipotropin. *Nature.* 260, 622-624.

- Li C.H., Yamashiro, Chung D., Doneen B.A., Loh H.H., Tseng L. 1976: Isolation, structure, synthesis and morphine like activity of β -endorphin from human pituitary glands. In ACTH and related peptides: structure, regulation and action. (Edit by Krieger D.T., Ganong W.F.) Annals of New York Acad. Sci. 297, 157-166.
- Li C.H., Rao A.J., Doneen B.A., Yamashiro D. 1977: β -endorphin: lack of correlation between the opiate activity and immunoreactivity by radioimmunoassay.
- Lightman S.L., Langdon N., Forsling M.L. 1980: Effects of the opiate antagonist naloxone and the enkephalin analog DAMME on the vasopressin response to a hypertonic stimulus in man. J. Clin. Endocrinol. Metab. 57, 1447-1449.
- Lindström L.H., Widerlöv E., Gunnar L.M., Wahlström A., Tirenus L. 1978: Endorphins in human CSF. Clinical correlations to some psychotic states. Acta Psychiat. Scand. 57, 153-154.
- Ling N., Burgus R., Guillemin R. 1976: Isolation, primary structure and synthesis of α -endorphin and γ -endorphin, two peptides of hypothalamic-hypophyseal origin with morphinomimetic activity. Proc. Natl. Acad. Sci. 73, 3942-3946.
- Liotta A.S., Suda T., Krieger D.T. 1978: β -lipotropin is the major opioid-like peptide of human pituitary and rat pars distalis: lack of significant β -endorphin. Proc. Natl. Acad. Sci. USA. 75, 2950-2954.
- Loh H.H., Tseng L.F., Wei E., Li C.H. 1976: β -endorphin is a potent analgesic agent. Proc. Natl. Acad. Sci. USA. 73, 2895-2898.
- Mains R.E., Eipper B.A., Ling N. 1977: Common precursor to corticotropin and endorphin. Proc. Natl. Acad. Sci. USA. 74, 3014-3018.
- McLoughlin L., Lowry P.J., Ratter S.J., Besser G.M., Rees L.H. 1980a: β -endorphin and β -MSH in human plasma. Clin. Endoc. 12, 287-292.
- McLoughlin L., Lowry P.J., Ratter S.J., Hope J., Besser G.M., Rees L.H. 1980b: Characterisation of the pro-opiocortin family of peptides in human cerebrospinal fluid. Neuroendocrinology. 32, 209-212.
- Miller R.J., Chang K.J., Cooper B., Cuatrecasas P. 1978: Radioimmunoassay and characterisation of enkephalins in rat tissues. J. Biol. Chem. 253, 531-538.
- Miller R.J., Pickel V.M. 1980: The distribution and function of the enkephalins. J. Histochem. Cytochem. 28, 903-917.
- Morley J.E., Barametsky N.G., Wingeit T.D., Carlson J.E., Heishman J.M., Milmed S., Levin S.R., Jamison K.R., Weitzman R., Chang R.J., Vainer A.A. 1980: Endocrine effects of naloxone-induced opiate receptor blockade. J. Clin. Endoc. Metab. 50, 251-257.
- Moult P.J.A., Grossman A., Evans J.M., Rees L.H., Besser G.M. 1981: The Effect of naloxone on pulsatile gonadotrophin release in normal subjects. Clin. Endocrinol. 14, 321-324.
- Pert C.B., Snyder S.H. 1973: Opiate receptor: demonstration in nervous tissue. Science. 179, 1011-1014.
- Pert C.B., Snyder S.H. 1974: Opiate receptor binding of agonists and antagonists effected differentially by sodium. Mol. Pharmacol. 10, 868-879.
- Pert C.B., Snowman A.M., Snyder S.H. 1974: Localisation of opiate receptor binding in synaptic membranes of rat brain. Brain Res. 70, 184-188.
- Phifer R.F., Orth D.N., Spicer S.S. 1974: Specific demonstration of the human hypophyseal adrenocorticotrophic (ACTH-MSH) cell. J. Clin. Endoc. Metab. 39, 684-693.
- Ratter S.J., Lowry P.J., Besser G.M., Rees L.H. 1980: Chromatographic characterisation of adrenocorticotrophin in human plasma. J. Endoc. 85, 359-369.
- Reid R.L., Hoff J.D., Yen S.S.C., Li C.H. 1981: Effects of exogenous β -endorphin on pituitary hormone secretion and its disappearance rate in normal subjects. J. Clin. Endoc. Metab. 52, 1179-1184.
- Roberts J.L., Herbert E. 1977: Characterisation of a common precursor to corticotropin and β -lipotropin: identification of β -lipotropin peptides and their arrangement relative to corticotropin in the precursor synthesised in a cell-free system. Proc. Natl. Acad. Sci. 74, 3052-3055.

- Ryder S.W., Eng J. 1981: Radioimmunoassay of leucine-enkephalin-like substance in human and canine plasma. *J. Clin. Endoc. Metab.* 52, 367-369.
- Sar M., Stumpf W.F., Miller R.J., Chang K.J., Cuatrecasas P. 1978: Immunohistochemical localisation of enkephalin in the rat brain and spinal cord. *J. Comp. Neurol.* 182, 17-37.
- Schützberg M., Lundberg J.M., Hökfelt T., Terenius L., Brandt J., Elde R.P., Goldstein M. 1978: Enkephalin-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. *Neurosci.* 3, 1169-1186.
- Seidah N.G., Chretien M. 1980: Complete amino acid sequence of a human pituitary glycopptide: An important maturation product of pro-opiomelanocortin. *Proc. Natl. Acad. Sci. USA.* 78, 4236-4240.
- Simantov R., Snyder H. 1976: Isolation and structure identification of a morphine-like peptide "enkephalin" in bovine brain. *Life Sci.* 18, 781-787.
- Simantov R., Kuh M.J., Uhl G., Snyder S.H. 1977: Opiate peptide enkephalin: immunohistochemical mapping in the rat nervous system. *Proc. Natl. Acad. Sci. USA.* 74, 2167-2171.
- Simon E.J., Hiller J.M., Edelman J. 1973: Stereospecific binding of the potent narcotic analgesic (3H) Emorphine to rat brain homogenate. *Proc. Natl. Acad. Sci. USA.* 70, 1947-1949.
- Sjölund B., Terenius L., Enkson M. 1977: Increased CSF levels of endorphin after electro-acupuncture. *Acta physiol. scand.* 100, 382-384.
- Smith R., Grossman A., Gaillard R., Clement-Jones V., Ratter S., Mallinson J., Lowry P.J., Besser G.M., Rees L.H. 1981: Studies on circulating met-enkephalin and β -endorphin: Normal subjects and patients with renal and adrenal disease. *Clinical Endoc.* 15, 291-300.
- Smyth D.G., Massey D.E., Zakassan S., Finnie M.D.A. 1979: Endorphins are stored in biologically active and inactive forms: isolation of alpha-N-acetyl peptides. *Nature.* 297, 252-255.
- Stern A.S., Lewis R.V., Kimura S., Rossier J., Stein S., Udenfriend S. 1980: Opioid hexapeptides and heptapeptides in adrenal medulla and brain. Possible implications on the bio-synthesis of enkephalins. *Arch. Biochem. Biophys.* 205, 606-613.
- Stubbs W.A., Delitalia G., Jones A., Jeffcoate W.J., Edwards C.R.W., Ratter S.J., Besser G.M., Bloom S.R., Alberti K.G.M.M. 1978: Hormonal and metabolic responses to an enkephalin analogue in normal man. *Lancet* ii, 1225-1227.
- Suda T., Liotta A.S., Krieger D.T. 1978: β -endorphin is not detectable in plasma from normal human subjects. *Science.* 202, 221-223.
- Sullivan S., Akil H., Watson S.J., Barchas J.D. 1977: Antibodies to enkephalins: coupling of antigens and a specific methionine-enkephalin radioimmunoassay. *Life. Sci.* 23, 121-126.
- Tan L., Yu P.H. 1980: Biosynthesis of enkephalins by chromaffin cells of bovine adrenal medulla. *Biochem. Biophys. Res. Commun.* 95, 1901-1908.
- Terenius L., Wahlstrom A. 1975: Search for an endogenous ligand for the opiate receptor. *Acta Physiol. Scand.* 94, 74-81.
- Teschmacher H., Opheim K.E., Cox B.M., Goldstein A. 1975: A peptide-like substance from pituitary that acts like morphine. I. Isolation. *Life Sci.* 16, 1771-1776.
- Tseng L.F., Loh H.H., Li C.H. 1976: β -endorphin as a potent analgesic by intravenous injection. *Nature.* 263, 239-240.
- Tseng L.F., Loh H.H., Li C.H. 1977: Human β -endorphin: development of tolerance and behavioural activity in rats. *Biochem. Biophys. Res. Commun.* 74, 390-396.
- Van Loon G.R., De Souza E.B., Ho D., Shin S.H. 1980: β -endorphin induced prolactin secretion is mediated by suppression of release of newly synthesised hypothalamic dopamine. *Can. J. Physiol. Pham.* 58, 436-439.
- Veshoeven W.M.A., Van Praag H.M., Ree J.M., van Wied D. de. 1979: Improvement of schizophrenic patients by treatment with (des-Tyrosine) γ -endorphin (DTYE). *Archs. gen. Psychiat.* 36, 294-298.
- Von Graffenreid B., Del Poyo E., Roubicek J., Krebs E., Pöldinger W., Burmeister P., Kerp L. 1978: Effects of the synthetic enkephalin analogue F.K. 33-824 in man. *Nature.* 272, 729.

- Wahlström A., Johansson L., Terenius L. 1976: Characterisation of endorphins (endogenous morphine-like fractions) in human cerebrospinal fluid and brain extracts. In: Kosterlitz H.W. (ed). *Opiates and endogenous peptides*. Amsterdam. Holland. 49-56.
- Wardlaw S.L., Frantz A.G. 1979: Measurement of β -endorphin in Human Plasma. *J. Clin. Endoc. Metab.* 48, 176-180.
- Wardlaw S.L., Wehrenberg W.B., Ferin M., Frantz A.G. 1980: β -endorphin stimulates prolactin in the intact, but not in the pituitary-stalk sectioned monkey. *Proc. 62nd Annual meeting of Endocrine Soc.* Washington D.C. p 246. (Abstract 688).
- Watson S.J., Berger P.A., Akil N., Mills M.J., Barchas J.D. 1978: Effects of naloxone on schizophrenia: reduction in hallucinations in a subpopulation of subjects. *Science*. 201, 73-76.
- Weber E., Voigt N., Martin R.: Concomitant storage of ACTH - and endorphin-like immunoreactivity in the secretory granules of anterior pituitary corticotrophs. *Brain Res.* 157, 385-390.
- Weitzman R.E., Fisher D.A., Minick S., Ling N., Guillemin R. 1977: β endorphin stimulates secretion of arginine vasopressin in vivo. *Endocrinol.* 101, 1643-1646.
- Wesche D., Höllt V., Hery A. 1977: Radioimmunoassay of enkephalins regional distribution in rat brain after morphine treatment and hypophysectomy. *Naunyn-Schmiedebergs' Arch. of Pharmacol.* 301, 79-82.
- Wiedemann E., Saito T., Linfoot J.A., Li C.H. 1977: Radioimmunoassay of human β -lipotropin in unextracted plasma. *J. Clin. Endoc. Metab.* 45, 1108-1111.
- Wouters W., Van Den Berkern J. 1980: Effects of met-enkephalin on slow synaptic inhibition in frog sympathetic ganglion. *Neuropharmacol.* 19, 237-243.
- Yamaguchi H., Liotta A.S., Krieger D.T. 1980: Simultaneous determination of human plasma immunoreactive β -lipotropin, γ -lipotrophin and β -endorphin using immuno-affinity chromatography. *J. Clin. Endoc. Metab.* 51, 1002-1008.
- Yang H.Y., Costa E., Di Giulio A.M., Fratta W., Hong J.S. 1979: Met-enkephalin-like peptides in bovine adrenal gland. *Fed. Proc.* 38, 364.

