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THE LIPOTROPHINS AND ENDORPHINS

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Isolation

βLPH

β-lipotrophin (βLPH) - 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 βLPH and termed γLPH (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 βmelanocyte stimulating hormone (βMSH). βMSH 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 an colleagues (1967) that βMSH was synthesised from βLPH with γLPH acting as an intermediate. However, in the human, this has been shown not to be the case, the βMSH 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 βMSH 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 antagonists 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 αMSH 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 untriakontapeptide 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 γendorphin (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 βLPH 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 βMSH (Abe et al. 1967; Donald and Toth 1973; Gilkes et al. 1975) were measuring β-LPH and γLPH or βMSH as a breakdown artefact of the assay system since it was confirmed that βMSH 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 ^{5}methionine 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 (γMSH) 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 γLPH and β-endorphin. However the cleavage products of the intermediate lobe show further processing to give the smaller peptides αMSH, CLIP ($ACTH^{17-39}$), γLPH 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 detemined (Seidah et al. 1981).

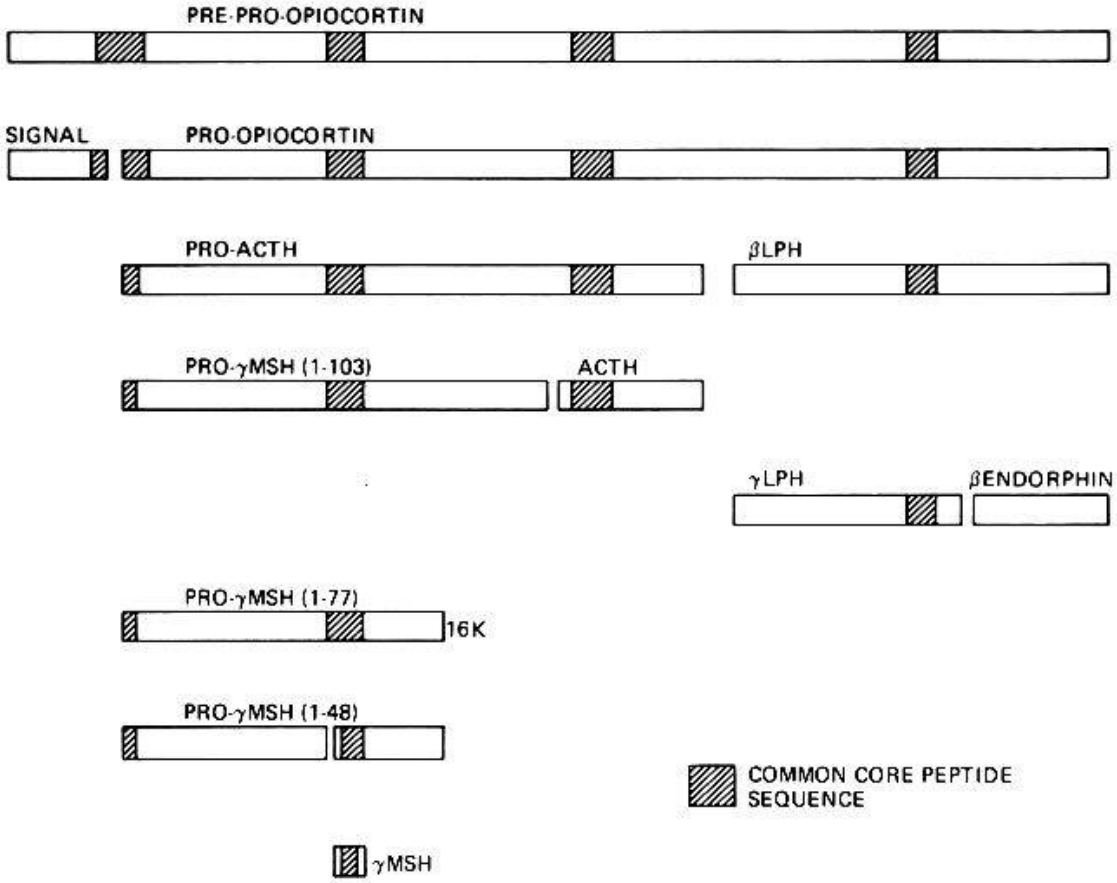

Fig. 1. Schematic representation of the relation of β-LPH, β-endorphin and ACTH to their common precursor pro-opiocortin.

Enkephalins

The appearance of the pentapeptide amino acid sequence of met-enkephalin in β-LPH (βLPH $^{61-65}$) 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

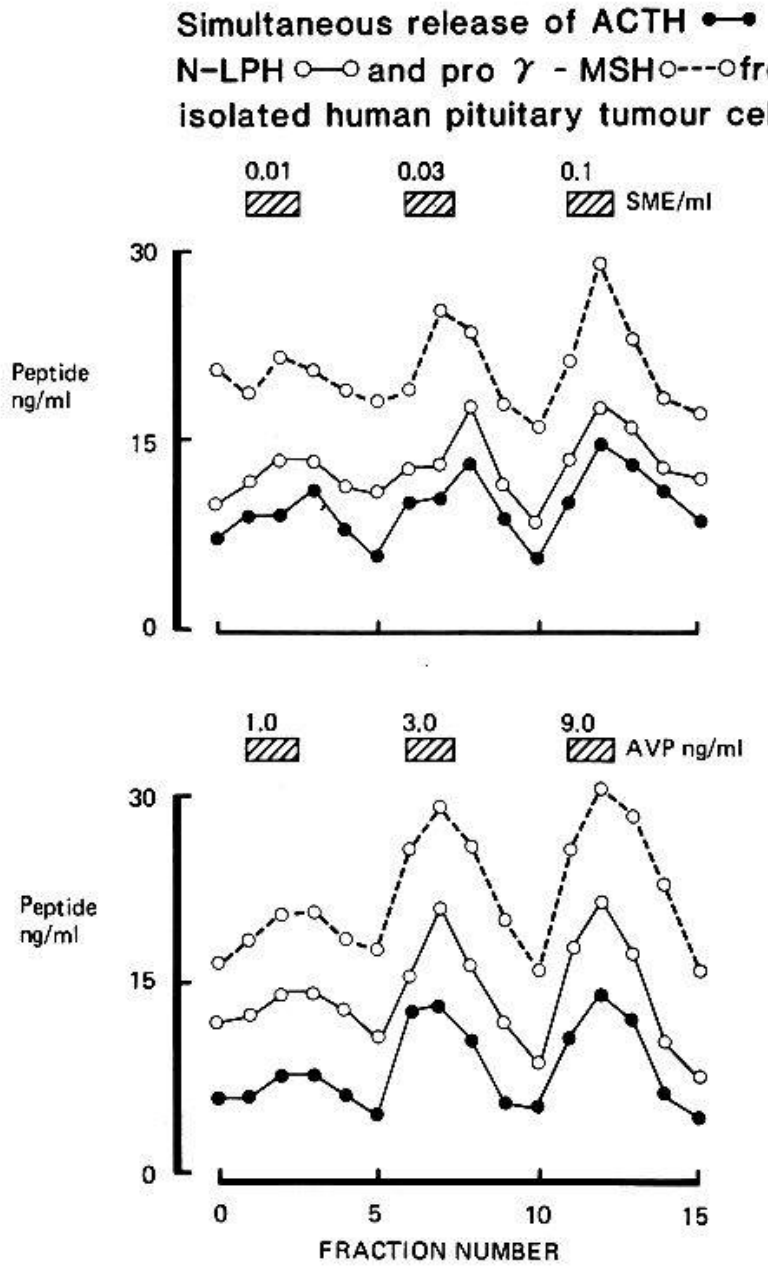

Fig. 2. Peptide release from isolated human pituitary tumour cells in response to: i) upper graph rat stalk median eminence extract (SME). ii) lower graph arginine vasopression
● —— ● ACTH 0 —— 0 N-LPH 0 ---- 0 pro-γ-MSH

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 hypothalami and called α-neo-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 γLPH. 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

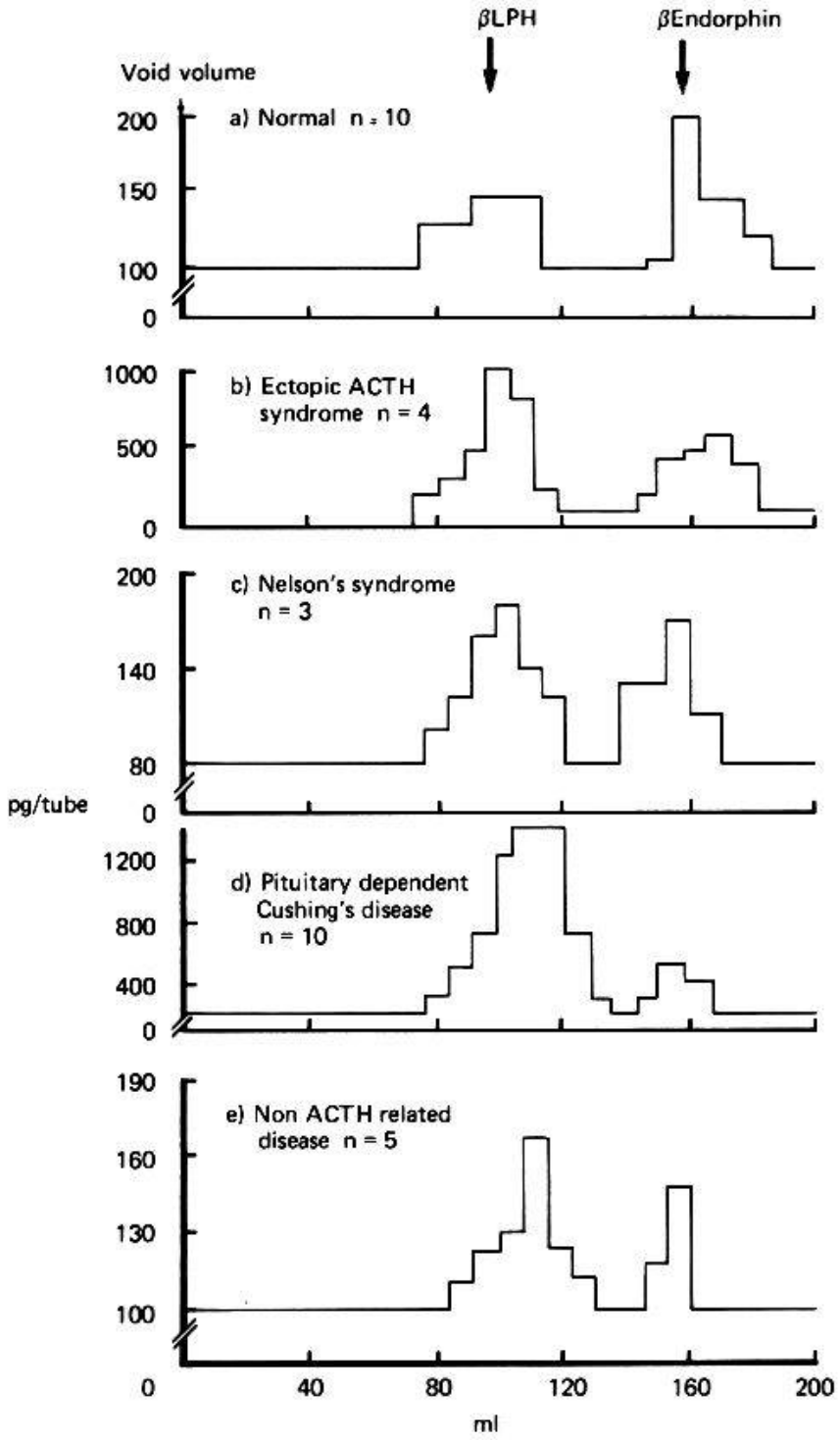

Fig. 3. Chromatographic profiles of C-terminal LPH activity in human plasma. Each profile is of one plasma sample representative of its group taken from normal subjects or patients with various disorders of the hypothalamic-pituitary-adrenal axis.

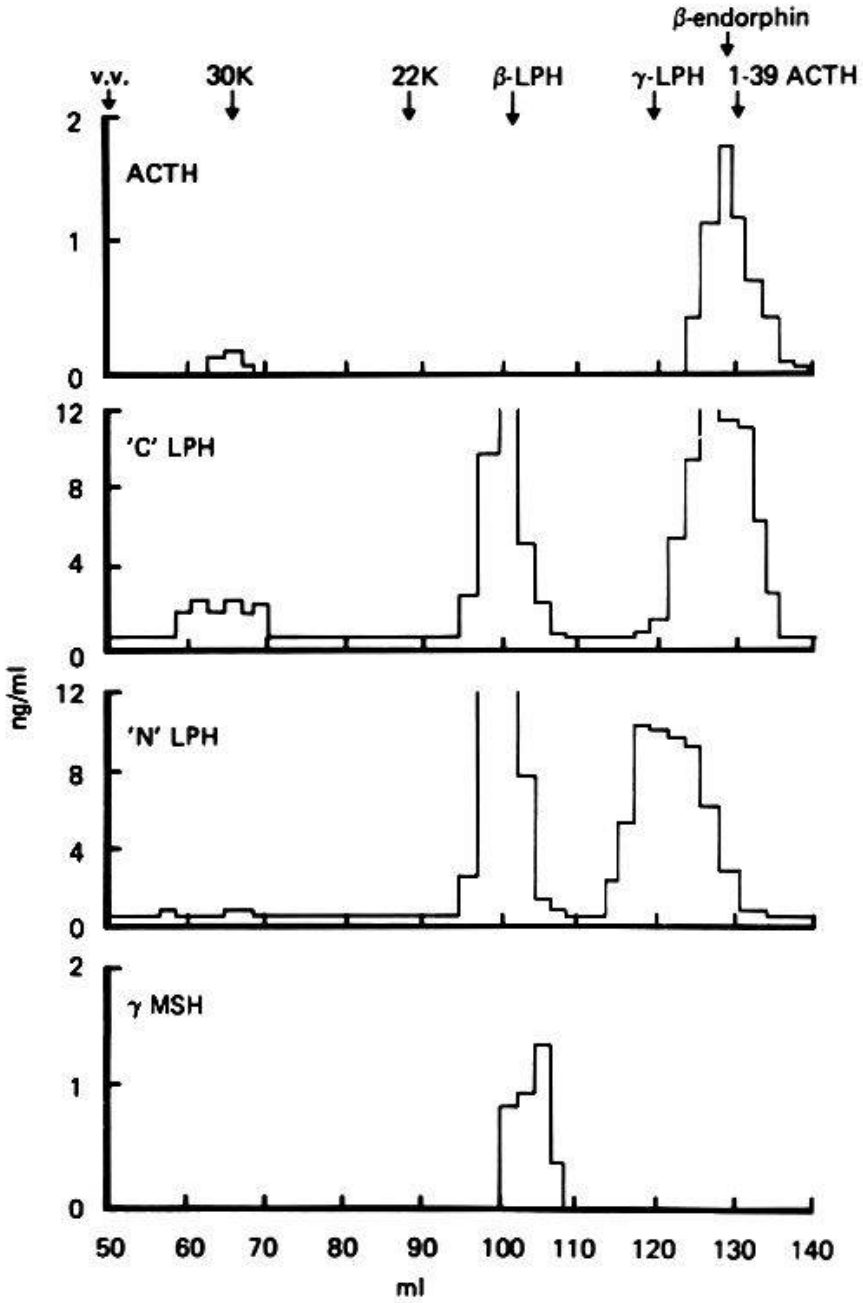

Fig. 4. Chromatographic profiles of ACTH, C-LPH, N-LPH and γ-MSH activity in pituitary tumour cell column effluent chromatographed on Sephadex G-75.

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, γLPH 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 methione 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 P_4 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, altough 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

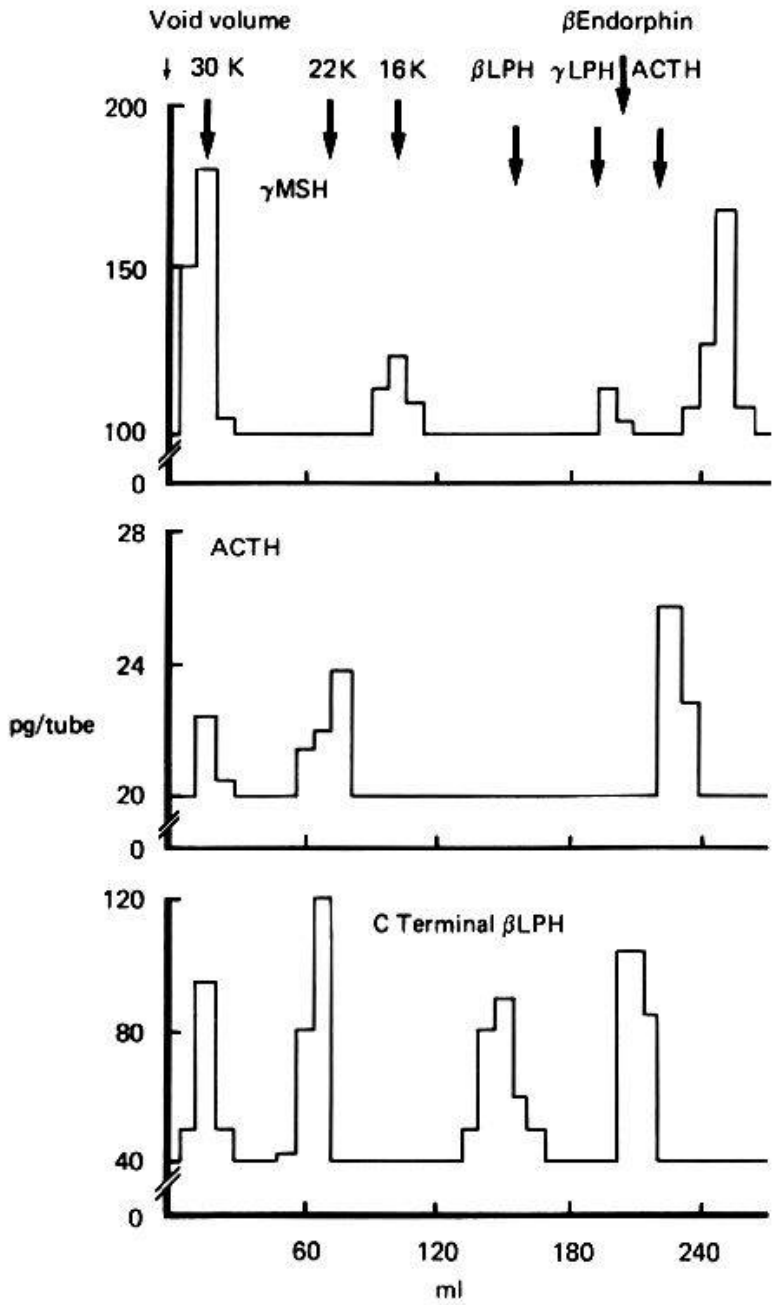

Fig. 5. Chromatographic profiles of pro-γ-MSH, ACTH and C-LPH activity in human CSF chromatographed on Sephadex G-75.

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, γLPH 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 panhypopituitarism. 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 widespread 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 (DTyE) 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 Graffenried 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 LRH 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.

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