Brain Opiates and Corticotrophin-related Peptides

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One of the most exciting discoveries of the last decade must be that of Hughes and his colleagues[1] who, in 1975, published in *Nature* their identification of two pentapeptides—methionine and leucine enkephalin—isolated from porcine brain and possessing potent opiate agonist activity. The prediction that opiate receptors of human brain must exist to recognise an endogenous analgesic thus found its way from fantasy to reality. Anecdotes from two world wars, relating severe injury with delayed pain perception, as well as those in happier times on the playing fields of England, had all pointed to the existence of an endogenous analgesic.

The elucidation of the relationship between these endogenous opiates and endocrinology begins with one of the most studied of all hormones, corticotrophin (ACTH). To understand this subject clearly it is first necessary to consider the techniques that have been used to study the physiology and pathophysiology of hormone secretion.

Hormone Measurement

Classically, hormones were measured by their function, and the use of such biological assay systems taught us most of what we know about the physiology and pathophysiology of hormone secretion. In recent years, with the advent of radioimmunoassay (RIA), it has become possible to measure hormones independently of their biological activity, such assays providing ‘structural’ rather than ‘functional’ information. Since the antisera used in RIA recognise a small group of amino acids that may be shared by peptides of quite differing sizes and functions, it may be necessary, for conclusive identification, to employ some method of separating them according to their size. Gel chromatography is one such technique, a sophisticated form of ‘molecular sieving’.

It was believed for many years that whenever ACTH was secreted it was accompanied by another peptide with potent pigmented activity, ‘β-MSH’[2]. Using a combination of the techniques mentioned above it was possible to disprove this long-held belief.

ACTH and ‘β-MSH’

It was always regarded as an enigma that human β-MSH, containing four extra amino acids in its N-terminal region, had 22 amino acids compared with the 18 in β-MSH molecules of other species. This observation, and other anomalies, led to a reappraisal of the biochemical characteristics of human β-MSH.

A study of the secretion of ACTH and β-MSH during the ‘stress’ of bacterial pyrogen administration in man was undertaken[3]; this preparation causes a rise in body temperature, rigors, and ACTH and immunoreactive β-MSH secretion. A study of the gel chromatography of blood taken during the height of ACTH secretion revealed that not all the circulating β-MSH molecules were the same size; some seemed to be much larger. Clearly, what was being measured in the β-MSH RIA was not the 22 amino acid peptide human β-MSH. However, it became obvious that the molecular size of this peptide was identical with that of a 91 amino acid peptide, which was first isolated from sheep pituitaries and called, rather inappropriately, β-lipotrophin (β-LPH) because of its weak ability to mobilise fat from adipocytes *in vitro*. The first 58 amino acids occur separately and are called γ-lipotrophin (γ-LPH), and the whole amino acid sequence of β-MSH is contained within both β- and γ-LPH (Fig. 1). Thus, the antiserum employed in the β-MSH

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Fig. 1. Schematic representation of proopiocortin, the precursor in man of γ-MSH, ACTH, β-LPH and β-endorphin. Further processing occurs in some species to form α-MSH, CLIP and possibly β-MSH. Met-enkephalin, although showing an amino acid sequence homology with β-LPH and β-endorphin, is derived from a separate precursor as yet unidentified[20]. (Courtesy Nature.)

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-120
γ-MSH

ACTH

β-LPH

α-MSH CLIP
γ-LPH
β-ENDORPHIN

β-MSH

MET-ENKEPHALIN?
RIA could, equally, be measuring β- or γ-LPH, or a combination of both; so direct measurement in blood without prior chromatography would not distinguish between these three peptides. So, in man, this assay was measuring β-LPH, not β-MSH, and a mistake in the original identification of the peptide must have occurred.

To re-examine this problem it is necessary to consider the conditions under which β-MSH was first isolated from human pituitaries[4]. The methods of extraction, utilising weak acid without the addition of proteolytic enzyme inhibitors, is likely to degrade β-LPH into smaller fragments. Repetition of this extraction procedure confirmed that proteolysis had taken place, with the artefactual production of β-MSH[5]. When human pituitary tissue was extracted under ideal conditions to minimise proteolysis, only β-LPH, not β-MSH, was present[6].

ACTH and β-LPH

Use of an antiserum measuring an amino acid sequence common to β-LPH and γ-LPH, but not contained within β-MSH, subsequently confirmed the presence of β-LPH in blood and that its secretion is associated with that of ACTH[7,8]. Thus, β-LPH secretion in man displays the characteristic circadian rhythm of ACTH, with a peak occurring in the early morning and a nadir around midnight. Since the biological role of secreted ACTH is believed to be its adrenal steroidogenic activity, we must now seek the biological role of β-LPH. Although β-LPH is weakly lipolytic and melanotropic, neither activity is believed to be of biological significance in man. Following the publication of the structures of the two peptides with potent opiate agonist activity, methionine and leucine enkephalin[1], it was recognised by Dr H. R. Morris that the amino acid sequence of met-enkephalin lay within the C-terminal region of β-LPH (see Fig. 1). It soon became clear that the whole of this region of β-LPH also had potent opiate agonist activity[9]; thus, the sequence of amino acids 61-91 of β-LPH was given the name β-endorphin—endorphin arising from the words 'endogenous morphine'.

β-LPH and β-Endorphin

It is immediately obvious that the structural similarity of β-LPH and β-endorphin means that most RIAs for β-endorphin could equally well measure β-LPH. However, this problem can be partly circumvented by using two antisera directed at different ends of the β-LPH molecule, an N-terminal antiserum which will measure β- and γ-LPH (N-LPH), and a C-terminal antiserum measuring β-LPH and β-endorphin (C-LPH).

When such measurements are applied to plasma taken at 9 a.m. from normal subjects, a fairly messy picture emerges, showing no consistent relationship between the amounts of N- and C-LPH. This suggests that β-endorphin is the predominant circulating peptide in some subjects, in others it is β-LPH alone, while a combination of all three peptides may occur in another group. Thus, in order to assert with confidence that β-endorphin circulates, it is again necessary to employ a chromatographic step before measurement with the two antisera. Using chromatography with the C-terminal antibody it has been shown that plasmas from normal subjects and patients with a variety of diseases of the pituitary-adrenal axis, such as Nelson's syndrome, the ectopic ACTH syndrome and Addison's disease, all contain both β-LPH and β-endorphin[10]. However, the origin of the circulating β-endorphin remains an enigma since, so far, all studies of adult postmortem human pituitary tissue clearly show the presence of β-LPH and γ-LPH but have failed to detect β-endorphin. It is interesting that β-endorphin has been found in fresh human fetal pituitary tissue; such a discrepancy could be explained by the loss of β-endorphin after death by postmortem autolysis in the adult.

In contrast, in vitro studies on surgical specimens of human pituitary adenoma tissue taken from adult patients with Cushing's disease or Nelson's syndrome do show release of β-LPH, γ-LPH and β-endorphin[11], and the control of secretion of all three peptides appears to be co-ordinated, as hydrocortisone can suppress their secretion, and a median eminence extract containing the putative corticotrophin releasing factor (CRF) can stimulate it. In vivo studies confirm these findings, ACTH and β-endorphin levels being suppressed in normal subjects after the administration of exogenous glucocorticoid in the form of dexamethasone.

In human CSF, the pattern of N- and C-LPH is much more distinct. Levels are ten times higher than in blood and, in contrast to blood, there is usually an excess of C-LPH over N-LPH, suggesting the consistent predominance of β-endorphin[12]. The origin of CSF β-endorphin is of considerable interest since, in a patient with panhypopituitarism and no detectable ACTH or LPH in blood, CSF levels are normal, showing the expected excess of C-terminal activity, chromatography again revealing the presence of β-endorphin[12]. In this instance it seems possible that the central nervous system is the source of CSF β-endorphin, and there is a considerable body of evidence showing that the brain itself is capable of synthesising ACTH, β-endorphin and related peptides.

Met-enkephalin

What then about the relationship between β-endorphin and the peptides isolated by Hughes and his colleagues, met- and leu-enkephalin? Attempts to measure these peptides are again fraught with the potential problems of cross-reactivity; antisera to met-enkephalin may cross-react with β-endorphin or β-LPH and with leucine enkephalin, as met- and leu-enkephalin differ by only one amino acid. An additional problem is the instability of met-enkephalin, which results in a tendency towards spontaneous oxidation of both endogenous and exogenous met-enkephalin in tissues, blood samples and during the laboratory procedure for its measurement. A radioimmunoassay has been developed to exploit this potential hazard, in which hydrogen peroxide is used to oxidise, in a controlled manner, all tissues and blood samples before
assay, creating a uniquely specific assay for met-enkephalin, which does not cross-react with β-LPH, β-endorphin or leu-enkephalin[13]. This RIA has been used in conjunction with an in vitro bioassay for opiate activity, in which opioid peptides inhibit the electrically-induced contraction of the mouse vas deferens, and with gel chromatography, as previously described. Using these combined techniques, a peptide identical to met-enkephalin has been identified in the human circulation for the first time[14].

Met-enkephalin circulates in normal subjects in concentrations ranging from 20 to 150 pg/ml. There is no circadian variation similar to that observed for ACTH and β-endorphin, and the concentration is not altered by exogenous glucocorticoids, which suggests that met-enkephalin secretion is unrelated to that of ACTH, β-LPH and β-endorphin and that it does not originate from the pituitary. Serendipity gave a clue to the origin of circulating met-enkephalin when a diagnostic venous catheterisation study was undertaken in a patient with an androgen-secreting adrenal tumour. It revealed a gradient of met-enkephalin in the left adrenal vein; levels in the internal jugular veins and peripheral veins of 55 and 53 pg/ml respectively were recorded, in contrast to levels of 116 and 101 pg/ml in the left adrenal vein and proximal left renal vein respectively. This suggests that the adrenal gland is one source of circulating met-enkephalin.

The normal adrenal cortex contains little met-enkephalin, but high levels occur in the adrenal medulla, some three times more than in the putamen of the brain, an area of high enkephalin content. However, much higher levels were found in two adrenal phaeochromocytomas, 20-50 times more than in the non-tumorous adrenal medulla[14]. Met-enkephalin was also demonstrated in the phaeochromocytomas by an immunofluorescent technique; intense fluorescence was observed in the cytoplasm of some cells, with little or none in others, presumably reflecting differing amounts of met-enkephalin.

Because of the high concentrations of met-enkephalin in the phaeochromocytomas, it was possible to obtain further biochemical characterisation, and chromatographic studies revealed met-enkephalin and a possible met-enkephalin precursor of larger size[15]. For the first time it was possible to purify enough met-enkephalin from one of these tumours and to obtain an amino acid analysis and sequencing, thus confirming that the tumour met-enkephalin was identical with the met-enkephalin first purified by Hughes and his colleagues from porcine brain[15]. It was also possible to confirm that this met-enkephalin had the expected biological activity in the in vitro opiate assay, and that this biological activity could be blocked by the specific morphine antagonist, naloxone. However, the larger form of met-enkephalin, a putative precursor, is devoid of biological activity and fails to produce opiate-like activity in the mouse vas deferens bioassay. But when it is treated with trypsin, bioactivity, reversible by naloxone, can be generated. As trypsin is classically used to cleave hormones from their precursors, this finding lends more weight to the supposition that the larger form of met-enkephalin is a true enkephalin precursor. Since the RIA for β-LPH or β-endorphin does not detect this precursor it must be unrelated to either peptide.

Enkephalin, Endorphin and Human Pathology

Ectopic Secretion

Both met-enkephalin and β-endorphin can be actively secreted by ectopic hormone-secreting tumours. In a study of one thymic carcinoid tumour secreting ACTH and associated with Cushing’s syndrome, a gradient of met-enkephalin was observed in the thymic vein, 132 pg/ml compared with 77 pg/ml simultaneously measured in a peripheral vein. Extracts of several such tumours demonstrated variable amounts of β-LPH, β-endorphin and met-enkephalin from tumour to tumour[16]. From a clinical standpoint, such peptides could theoretically suppress local pain production by the tumour and, since they produce behavioural and mood changes in animals, they could also produce psychiatric disturbances in man. Furthermore, the production of these peptides may be only the tip of the iceberg; many tumours may produce a variety of psychoactive substances that could be responsible for some of the varied and unexplained clinical syndromes associated with malignant disease, including psychiatric disturbances in the absence of cerebral metastases.

Acupuncture

Acupuncture analgesia has been employed for centuries in China and recent studies in the West have confirmed that pain thresholds increase after acupuncture. Although the mechanism of action is uncertain, the delay in the onset of analgesia, the prolonged and persistent effects, and the report that analgesia can be produced in recipient animals after transfer of post-acupuncture CSF, all provide evidence of the release of a humoral factor. Since acupuncture analgesia can be reversed by the specific opiate antagonist, naloxone, it seems likely that endogenous opiates are involved in some way.

In 1975, Dr H. L. Wen, a neurosurgeon in Hong Kong, reported that the symptoms of heroin withdrawal could be alleviated by electro-acupuncture and a collaborative study was undertaken with him in which blood and CSF levels of β-endorphin and met-enkephalin were measured in heroin addicts undergoing treatment in his unit. The results obtained were contrary to the expectation that heroin addiction would suppress β-endorphin levels and that this suppression would be responsible for the withdrawal symptoms. In fact, β-endorphin levels in blood and CSF were higher than those normally observed, and these high levels of β-endorphin could not be altered by the electro-acupuncture. In contrast, met-enkephalin levels in the plasma and CSF of the addicts were normal, but CSF met-enkephalin levels rose significantly in all patients after successful electro-acupuncture[17] (Fig. 2).

In a further study with Dr Wen, the basal levels of β-endorphin and met-enkephalin in the lumbar CSF of
patients having electro-acupuncture for recurrent pain were observed to be no different from those in pain-free control subjects. However, after electro-acupuncture in the patients with pain, significant increases in CSF β-endorphin occurred in all subjects, but met-enkephalin levels were unchanged[18] (Fig. 3). These results suggest that the analgesia observed after electro-acupuncture in patients with recurrent pain may be mediated by release into the CSF of β-endorphin, in contrast to the relief of heroin withdrawal symptoms that is related to met-enkephalin release.

It has recently been reported that naloxone completely reversed the electro-acupuncture analgesia of low frequency, but not high frequency, stimulation[19], suggesting that acupuncture analgesia could be mediated by at least two pain-relieving mechanisms, one which is reversible by naloxone and related to β-endorphin, and the other, which can be reversed by the serotonin synthesis inhibitor, parachlorophenylalanine, related to serotonin. The evidence would support an endorphin-related mechanism in patients receiving low frequency electro-acupuncture for recurrent pain, and an enkephalinergic system in the heroin addicts experiencing relief during heroin withdrawal.

**ACTH/β-LPH/β-Endorphin**

In 1979, Nakashima and his co-workers[20] predicted the full amino acid sequence of the bovine ACTH/LPH precursor (see Fig. 1), by nucleotide sequencing of cDNA. From their achievement we know that the pituitary contains a glycopeptide with a molecular weight of 31,000, which contains the complete amino acid sequence of ACTH joined to β-LPH. Most available evidence supports the theory that β-LPH exists primarily as a precursor of the opiate active peptide, β-endorphin. All three peptides, ACTH, β-LPH and β-endorphin are secreted in response to stress, possibly to serve an adaptive function, with β-endorphin aiding pain relief. Methionine-enkephalin does not derive from the 31,000 molecular weight precursor and is therefore not related to β-endorphin, although its precursor awaits identification.

Overall, the data suggest that enkephalins and endorphins may be mobilised to modulate pain perception in certain conditions, and such observations provide a physiological basis for the known biological variations in response to pain, often embodied in phrases such as 'attitudes of mind' or 'low pain threshold'.

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Money Money Money

Money is no new problem for the College. There was that murky affair of Dr Daniel Whistler and Sir John Cutler, mentioned in the Annals two days after Whistler's death. 'At an extraordinary meeting, there was a consultation about the outstanding peculation of Daniel Whistler ... at which the most noble and prudent Sir John Cutler, Baronet, was present.' Dr A. M. Cooke cleared Whistler's reputation in 1967 (J. Roy. Coll. Physcs London, 1, 221), finding him careless and no match for the financial skills of Cutler. Anyway, the affair left the College's finances in a tangle. So an entry in the Annals for 1690 casts an odd light on possible income. 'Dr John Hutton, Chief Physician to King William admitted a Fellow ... He indeed, lest he should seem to receive so outstanding a mark of honour ungratefully, gave the College a sufficiently honourable amount of gold and undertook also (if everything went according to promise) to bestow a more valuable gift on them eventually.' A little later comes an entry recording the death of that most distinguished physician and physiologist, Dr Richard Lower. The entry goes on 'In medical practice and to a great extent among the legations he had amassed a considerable estate which he had willed very liberally to various purposes. To the College of Physicians of London he left not a penny. One may wonder how I can tell of a Fellowship hitherto so outstandingly jealous for the common good yet of such unfriendliness to his colleagues.' The Registrar of that time, for one year only, Dr Richard Griffith, may have been jealous of Lower's outstanding career as a pioneer of pulmonary physiology (he experimented with blood transfusions; 'I shall try it on a dog and I shall get a tin pipe made') or because Lower left £1,000 to Barts. He left a similar sum to French and Irish Protestant refugees. Poor Lower died of a chill contracted while extinguishing a fire in his chimney. For some unknown reason the Registrar contrasted, in the same entry in the Annals, Lower's career with that of Dr William Hawkins, who died at the same time. 'A man of exceptional scholarship and graceful build, polished in manners and very successful in medical practice, but so exceedingly poor that ... he was buried at the public expense of his colleagues and friends. Throughout his life a striking example of human frailty ... he lacked no quality but prudence.' The President of that time, Dr Charleton, also went broke and retired for a while to Jersey. No wonder they all worried about money.