Ectopic production of methionine enkephalin and beta-endorphin

P T PULLAN, VICKY CLEMENT-JONES, R CORDER, P J LOWRY, G M REES, LESLEY H REES, G M BESSER, M M MACEDO, A GALVAO-TELES

Summary and conclusions

Immunoreactive methionine enkephalin and beta-endorphin were sought by serial dilution of tissue extracts and assay of chromatographic fractions in non-endocrine tumour tissue from three patients with the ectopic adrenocorticotrophin syndrome associated with carcinoid tumours and in normal lung tissue and thymic tissue from a patient with myasthenia gravis. In all cases serial dilution of extracts showed parallelism to standard radioimmunoassay curves.

The two peptides were found in high concentration in the three tumours but were undetectable in the control tissues. In a single case tested the methionine enkephalin concentration in a vein draining the tumour was twice that in a peripheral vein.

In view of their profound effects on behaviour in animals and potent analgesic activity in animals and man the ectopic secretion of methionine enkephalin and beta-endorphin may modify the clinical features of a wide variety of tumours and produce some of the diverse clinical syndromes associated with malignancy.

Introduction

The synthesis and secretion of adrenocorticotrophin (ACTH) from non-endocrine tumours may lead to the clinical picture of Cushing's syndrome. The condition is associated with ectopic lipotrophin secretion.[1] ACTH and lipotrophin are synthesised via a common precursor molecule,[2] and the recently discovered opioid peptides β-endorphin and methionine enkephalin share a common amino-acid sequence with the C-terminal portion of the β-lipotrophin molecule.[3] We therefore decided to investigate the possibility of ectopic production of these peptides in patients with non-endocrine tumours secreting ACTH.

Patients and methods

We studied three women with the ectopic ACTH syndrome. The first patient (case 1), aged 39, presented in April 1973 with a manic psychosis and typical physical appearance of Cushing's syndrome. The diagnosis was confirmed biochemically, and over the next four years the disease took a cyclical course both clinically and biochemically and spontaneously remitted and recurred several times. In December 1977 a malignant thymic carcinoid tumour about 5 cm in diameter was discovered and removed. The second patient (case 2), aged 44, presented in January 1976 with depression and features of Cushing's syndrome, which was confirmed biochemically. A thymic carcinoid tumour 3 cm in diameter was discovered and removed in March 1979. The third patient (case 3), aged 37, presented in July 1979 with an 18-month history of Cushing's syndrome associated with depression. The diagnosis was confirmed biochemically, and in September a pulmonary carcinoid tumour 1 cm in diameter was removed from the left lower lobe. In all cases the psychiatric disturbances and Cushing's syndrome disappeared with removal of the tumours.

Tumour tissue obtained at operation was immediately frozen on dry ice and stored at −70°C until extraction and assay. The tissue was homogenised in 0·1M HCl and after neutralisation was assayed in serial dilution for immunoreactive β-endorphin (C-terminal β-lipotrophin)[4] and methionine enkephalin.[5] The second assay is specific for methionine enkephalin, whereas the β-endorphin assay shows complete molar cross-reactivity with β-lipotrophin. Chromatography of fresh tumour extracts was performed on a 1·5 × 100 cm column of Sephadex G-75 equilibrated with 1% formic acid containing 1 g Polypep (Sigma, P5115) and pumped upwards at 2 ml/h at 4°C, 1·8 ml fractions being collected. An aliquot of each fraction was then neutralised by dilution in assay buffer and assayed for β-endorphin and methionine enkephalin. The column was calibrated with human β-lipotrophin purified in this laboratory and with synthetic β-endorphin and methionine enkephalin (Bachem). This system of chromatography prevents β-endorphin and methionine enkephalin being generated as artefacts from β-lipotrophin.[5,6] Fresh control tissue obtained at operation from two patients without the ectopic ACTH syndrome was processed in the same manner as the tumours. Normal lung tissue was obtained from a patient with a pulmonary carcinoid tumour and thymic tissue from a patient with myasthenia gravis.

In case 2 blood samples taken simultaneously from a vein draining the tumour and from a peripheral vein were analysed for methionine enkephalin concentrations after extraction.[5]

Results

The table shows the concentrations of immunoreactive β-endorphin and methionine enkephalin in the three tumours and control tissue expressed in μg/g wet weight tissue. In all cases serial dilution of tumour extracts showed parallelism to the standard radioimmunoassay curves. β-endorphin and methionine enkephalin were undetectable in normal lung tissue and thymic tissue from the patient with myasthenia gravis. Assay of the chromatographic fractions for β-endorphin (see figure: left) showed a peak of immunoreactivity eluting in the position of β-endorphin in all three tumours, but a peak in the position of β-lipotrophin could not be detected in tumour 2. The methionine enkephalin assay (figure: right) showed a peak coeluting with synthetic methionine enkephalin, and peaks of immunoreactive materials of larger molecular weight were also present.

In case 2 the plasma methionine enkephalin concentrations in a vein draining the tumour and simultaneous peripheral venous sample were 132 ng and 77 ng/l respectively.

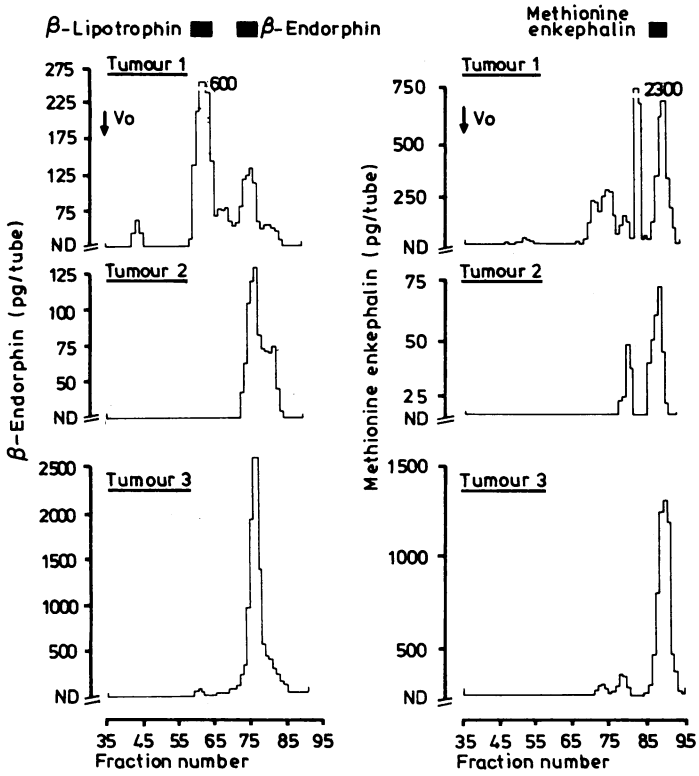
Concentrations of β-endorphin (C-terminal β-lipotrophin) and methionine enkephalin in carcinoid tumours and control tissues

	Tumour			Control tissue	
	1	2	3	Lung	Thymus
β-Endorphin (μg/g wet weight tissue)	179·0	18·0	1818·0	<0·01	<0·01
Methionine enkephalin (μg/g wet weight tissue)	0·4	4·0	1·0	<0·04	<0·04

St Bartholomew's Hospital, London EC1A 7BE
P T PULLAN, PHD, FRACP, research fellow
VICKY CLEMENT-JONES, MB, MRCP, MRC training fellow
R CORDER, BSC, research assistant
P J LOWRY, PHD, senior lecturer in endocrinology
G M REES, MS, FRCS, consultant cardiac surgeon
LESLEY H REES, MD, FRCP, professor of chemical endocrinology
G M BESSER, MD, FRCP, professor of endocrinology

Lisbon City Hospital, Hospital de Santa Maria, 1200 Lisbon, Portugal
M M MACEDO, MD, director, department of cardiothoracic surgery

Santa Maria's University Hospital, 1600 Lisbon, Portugal
A GALVAO-TELES, MD, PHD, professor of medicine



Sephadex G-75 column chromatography of tumour extracts from three women with ectopic ACTH syndrome. Left: Fractions assayed for β-endorphin (C-terminal β-lipotrophin). Right: Fractions assayed for methionine enkephalin. Figure shows positions of purified human β-lipotrophin and synthetic human β-endorphin and methionine enkephalin. Vo = Void volume determined by spectrophotometry. Baseline is limit of detection in assay at dilution of fraction. ND = Not detectable.

Discussion

Ectopic β-endorphin secretion was described in a patient with pancreatic islet-cell carcinoma.[7] Our patients are the first reported cases in which methionine enkephalin has been found in non-endocrine tumours, and in all three this was associated with β-endorphin. These peptides were undetectable in normal lung tissue and thymic tissue from a patient with myasthenia gravis, suggesting that their presence in carcinoid tumours derived from these tissues was ectopic. That the methionine enkephalin concentration in blood from a vein draining the tumour was roughly twice that in a peripheral vein suggests that methionine enkephalin was secreted by this tumour. Enkephalin has been reported in an adrenal phaeochromocytoma and an adrenal ganglioneuroma,[8] but these may not be classed as instances of ectopic enkephalin production as this peptide is normally found in the adrenal medulla.[9]

Finding β-endorphin and methionine enkephalin in non-endocrine tumours may have important clinical implications. These opioid peptides have profound behavioural effects on laboratory animals, including the production of a catatonic-like state,[10 11] and it is tempting to speculate that secretion of these peptides by the tumours could have been at least partly responsible for the psychiatric disturbances in our patients. Depressive illness may precede other clinical features of malignancy by several years,[12 13] and at the 60th annual meeting of the Endocrinology Society in 1978 (abstract No 100) C E Jackson *et al* reported that medullary carcinoma of the thyroid (which often secretes peptide hormones) is associated with a high incidence of depression before the patients have any knowledge of a thyroid abnormality. β-endorphin and methionine enkephalin also have potent analgesic activity.[13 15] Possibly their secretion by the tumour and the associated central psychological effects suppressed some of the clinical features in our patients, including pain from the tumour. This is supported by the considerable delay between the onset of Cushing's syndrome and detection of the tumour (4·7 years in case 1, 3·2 years in case 2, and 1·5 years in case 3).

Finally, that many (if not all) small-cell lung carcinomas contain ACTH[16] suggests that ectopic production of the related peptides β-endorphin and methionine enkephalin may be common, and probably this is associated with release of other peptides with behavioural effects. These centrally active, ectopically secreted neuropeptides may combine to modify the clinical features of a wide variety of tumours, producing some of the diverse clinical syndromes associated with malignancy.

PTP is in receipt of a Florey fellowship and an overseas travelling fellowship of the Royal Australasian College of Physicians. We thank the Cancer Research Campaign, Medical Research Council, Wellcome Trust, and Joint Research Board of St Bartholomew's Hospital for support, and Professor I Doniach and Dr A G Standsfeld for interpreting the histology. Mrs E Penman kindly collected tumour 1 from Lisbon.

Requests for reprints should be sent to Professor G M Besser.

References

1. Gilkes JJH, Rees LH, Besser GM. Plasma immunoreactive corticotrophin and lipotrophin in Cushing's syndrome and Addison's desease. *Br Med J* 1977;i:996-8.
2. Mains RE, Eipper BA, Ling NR. Common precursor to corticotrophins and endorphins. *Proc Natl Acad Sci USA* 1977;**74**:3014-8.
3. Goldstein A. Opioid peptides in pituitary and brain. *Science* 1976;**193**: 1081-6.
4. Jeffcoate WJ, McLoughlin L, Rees LH, *et al.* β-Endorphin in human cerebrospinal fluid. *Lancet* 1978;ii:119-21.
5. Clement-Jones V, Lowry PJ, Rees LH, Besser GM. Development of a specific extracted radioimmunoassay for methionine enkephalin in human plasma and cerebrospinal fluid. *J Endocrinol* (in press).
6. McLoughlin L, Lowry PJ, Ratter SJ, Besser GM, Rees LH. β-Endorphin and β-MSH in human plasma. *Clin Endocrinol (Oxf)* (in press).
7. Orth DN, Guillemin R, Ling N, Nicholson WE. Immunoreactive endorphins, lipotrophins and corticotrophins in a human non-pituitary tumor: evidence for a common precursor. *J Clin Endocrinol Metab* 1978;**46**: 849-52.
8. Sullivan SN, Bloom SR, Polak JM. Enkephalin in peripheral neuroendocrine tumours. *Lancet* 1978;i:986-7.
9. Clement-Jones V, Lowry PJ, Rees LH, Besser GM. Metenkephalin circulates in human plasma. *Nature* 1980;**283**:295-7.
10. Bloom F, Segal D, Ling N, Guillemin R. Endorphins: profound behavioural effects in rats suggest new aetiological factors in mental illness. *Science* 1976;**194**:630-2.
11. Jacquet YF, Marks N. The C-fragment of β-lipotrophin: an endogenous neuroleptic or antipsychotogen ? *Science* 1976;**194**:632-5.
12. Fras I, Litin EM, Pearson JS. Comparison of psychiatric symptoms in carcinoma of the pancreas with those in some other intra-abdominal neoplasms. *Am J Psychiatry* 1967;**123**:1553-62.
13. Kerr TA, Schapira K, Roth M. The relationship between premature death and affective disorders. *Br J Psychiatry* 1969;**115**:1277-82.
14. Loh HH, Tseng LF, Wei E, Li CH. β-Endorphin is a potent analgesic agent. *Proc Natl Acad Sci USA* 1976;**73**:2895-8.
15. Belluzi JD, Grant N, Garsky V, Sarantakis D, Wise CD, Stein L. Analgesia induced in vivo by central administration of enkephalin in the rat. *Nature* 1976;**260**:625-6.
16. Bloomfield GA, Holdaway IM, Corrin B, *et al.* Lung tumours and ACTH production. *Clin Endocrinol (Oxf)* 1977;**6**:95-104.

(*Accepted 2 January 1980*)

ONE HUNDRED YEARS AGO At an asylum in Vienna (says the *Danube*), a novel method of treatment has been adopted. The director has established a lithographed journal for circulation in the asylum, and he induces the patients to contribute to it. Especially he encourages them to refute the manias of their comrades. The man who believes his nose to be made of sugar-candy, and liable to dissolve, he says, can argue with excellent logic against the folly of his friend's theory that his beard is a tender plant and needs frequent watering. As a rule, they are able to discuss with good sense all subjects except those which concern their peculiar delusion. (*British Medical Journal*, 1880.)