Pergamon

0960-0760(95)00044-5

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 139–151, 1995

0960-0760/95 $9.50 + 0.00

Ectopic ACTH Syndrome

Bernardo L. Wajchenberg,[1]* Berenice B. Mendonça,[1] B. Liberman,[1] Maria Adelaide A. Pereira[1] and Marvin A. Kirschner[2]

[1]Endocrine Service, Hospital das Clínicas of The University of São Paulo School of Medicine, Av. Dr Eneas Carvalho de Aguiar 255, 05403-000 São Paulo, Brazil and [2]Department of Medicine, Newark Beth Israel Medical Center, Newark, NJ, U.S.A.

Ectopic ACTH syndrome represents a cancer-induced amplification of a property [proopiomelanocortin (POMC) peptides production] normally present in the cells from which the cancer originated but with aberrant posttranslational processing of POMC resulting in a greatly elevated secretion of ACTH precursors. The classic ectopic ACTH-producing tumors described in the 1960s were highly malignant but more recently slowly growing tumors such as carcinoids are reported with increasing frequency. Clinical features of patients with ectopic ACTH were analyzed, including biochemical abnormalities, plasma ACTH, cortisol and urinary steroids. Dynamic tests such as high-dose dexamethasone suppression, metyrapone and ovine-CRH (oCRH) stimulation were explored, as well as inferior petrosal sinus ACTH sampling before and after oCRH. Among the tumor markers examined, elevation of ACTH precursors was uniformly present followed by increased output of calcitonin, gut hormones, oncofetal and placental hormones in decreasing order. Since more than 90% of ectopic ACTH tumors are neuroendocrine in nature exhibiting APUD characteristics, their 2 markers, neuron-specific enolase and chromogranins are very useful. The imaging procedures for localization of the tumor ranged from chest X-rays to computed tomography and magnetic resonance of the chest and abdomen. Abdominal ultrasonography was also useful. Finally somatostatin receptor scintigraphy permitted demonstration of unrecognized tumors and/or metastases, even when the tumors were occult. The ACTH content, immunostaining for APUD markers and altered POMC processing were evaluated in ectopic tumors and/or metastases. Occult ectopic ACTH syndrome of more than 4–6 months of symptoms without the emergence of an obvious source was reviewed. Since the tumors are often clinically and biochemically undistinguishable from pituitary-dependent Cushing's disease, inferior petrosal sinus sampling for ACTH after oCRH stimulation established the diagnosis in over 90% of the cases. 60% of the occult tumors were thoracic carcinoids (3/4 bronchial carcinoids), followed by small cell lung cancer and pancreatic neuroendocrine tumors. In 12% the primary etiology was not detected. The rare syndrome of ectopic CRH syndrome (6 published cases) leading to excessive stimulation of the pituitary which became hyperplastic and secreted excessive amounts of ACTH is discussed. Finally, the 12 published cases and 1 unreported patient with ectopic CRH-ACTH tumors were reviewed, the majority being metastatic small cell lung carcinomas, bronchial and thymic carcinoids.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1–6, pp. 139–151, 1995

INTRODUCTION

It has become apparent during the last decades that a wide range of endocrine tumors secrete hormones not normally associated with the gland in which the neoplasm arises and that nonendocrine tumors can synthesize and/or secrete polypeptide hormones and other tumor-associated proteins. The production of such tumor markers has come to be refered to as "ectopic" when the marker is not associated with the tissue from which the tumor derives. With the use of present techniques of hormone detection these syndromes are felt to be common. Indeed, Odell *et al.* [1] have postulated that all tumors endocrine or not, make proopiomelanocortin (POMC), an ACTH precursor, and other protein markers. Thus, hormonal endocrine syndromes are not manifestations of "ectopic hormone production" but represent cancer-induced

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
*Correspondence to B. L. Wajchenberg.

amplification of a property that is normally present in the cells from which the cancer originated. Nevertheless, the term "ectopic hormone syndromes" occurring with nonendocrine neoplasms is well established and widely used. It should be apparent, however, that most ACTH-secreting tumors are indeed endocrine tumors.

All tissues synthesize small amounts of POMC-derived peptides (probably paracrine or autocrine messengers for the tissues) and in much greater quantities by most carcinomas, due to the enhanced rate of POMC gene transcription or the inability to prevent continuous expression of the POMC gene. The persistent synthesis and secretion of POMC-derived peptides is associated with a tendency to process the POMC peptide product incompletely [2, 3].

The ratio of POMCmRNA/POMC-peptides concentrations is many times greater in nonpituitary tumors than in the pituitary gland and is probably related to the POMCmRNA being translated into an alternate reading frame (promoter switching) qualitatively modifying the expression and compromising the tumor's ability to secrete regular POMC-peptides at the posttranscriptional level. Other possibilities could be a non-efficient mRNA translation or rapid release and/or degradation of POMC [4]. Thus, ectopic ACTH syndrome is characterized by over-expression and aberrant posttranslational processing of POMC. The elevation of ACTH precursors (POMC and proACTH) but not ACTH itself correlate with plasma cortisol in ectopic ACTH [3]. Gross elevation of precursors despite minimal bioactivity can be sufficient to induce severe hypercortisolemia [3].

Finally, non-pituitary ACTH-secreting tumors are generally unresponsive to both CRH (corticotropin-releasing hormone) and glucocorticoids [5].

PREVALENCE OF CUSHING'S SYNDROME ASSOCIATED WITH ECTOPIC ACTH

In all large series of patients with Cushing's disease there was a preponderance of pituitary ACTH dependent Cushing's disease, ranging from 55 to 82% of cases. Ectopic ACTH syndromes represented 9–18% of the patients in 4 large series: 88 patients from St Bartholomew's Hospital in London [6]; 312 patients from the Mayo Clinic, Rochester [7]; 254 patients from the National Institutes of Health, Bethesda [8] and 158 patients reported from Hospital das Clínicas, São Paulo [9]. By contrast to the earlier large series of ectopic ACTH-producing neoplasms with a predominance of lung carcinomas, the more recent series show a predominance of "benign" tumors, namely thoracic carcinoids (Table 1; Refs [6, 9–12]) probably due to the fact that clinically apparent lung cancers are not reported any more. On the other hand, in the majority of patients in the newer series the diagnosis of ACTH-secreting neoplasm became evident only after months and years following the onset of hypercortisolism. Such patients were generally referred to Endocrine Clinics as diagnostic dilemmas.

Regarding the types of neoplasms producing ACTH, small cell lung carcinomas were the single more common cause of Cushing's syndrome reported in the earlier series (Table 1) although most of these were not associated with Cushing's syndrome. Subtle abnormalities of cortisol secretion may exist in these patients in the absence of overt clinical findings. In the most recent series, besides bronchial and thymic carcinoids, pancreatic carcinomas and pheochromocytomas are well represented. It should be noted that tumors arising from mesodermal tissue (i.e. sarcomas) have not been reported to make ACTH.

Almost all tumor types that produce ACTH also secrete other hormones, such as gastrin in sporadic Zollinger–Ellison syndrome, catecholamines and calcitonin in pheochromocytomas and related tumors. The miscellaneous group of ectopic ACTH producing tumors includes neoplasms which uncommonly produce ACTH, even though the tumors themselves are extremely common.

The majority of the tumors that give rise to the ectopic ACTH syndromes usually fall into one of 4 histopathological types: small cell lung carcinomas, carcinoids, pancreatic islet cell tumors and pheochromocytomas, all of them now well recognized to arise

Table 1. Types of neoplasms causing ectopic ACTH syndrome (values are percentage of cases)

	Liddle *et al.*, 1969 [10] (104 patients)	Imura *et al.*, 1978 [11] (51 patients)	Jex *et al.*, 1985 [12] (21 patients)	Howlett *et al.*, 1986 [6] (16 patients)	Doppman *et al.*, 1989 [13] (28 patients)	Wajchenberg *et al.*, 1994 [9] (12 patients)
Ca lung	50	49	20	19	—	8
Pancreatic cystadenoma	—	—	4	—	—	—
Miscellaneous Ca or hematologic Ca	10	10	—	12	—	—
Adeno Ca, undetermined	7	2	8	—	32	—
Bronchial adenoma (carcinoid)	5	8	28	37	39	17
Ca thymus, thymic carcinoid	10	12	8	12	7	25
Ca pancreas (incl. carcinoid & islet cell)	10	6	16	12	11	25
Pheochromocytoma	3	2	12	6	11	25
Medullary Ca thyroid	2	6	—	—	—	—
GI carcinoid	—	6	—	—	—	—

from neuroendocrine tissues, underlying the close association between the neural and endocrine systems. The classic work of Pearse [14] identified these tumors as exhibiting APUD (amine precursor uptake and decarboxylation) characteristics: peptides and/or biogenic amines and precursors secretion, presence of storage granules, possessing chromogranin and enolase activities. Many experimental studies have led to the realization that APUD characteristics within some cells and the ability to produce hormones in normal tissues do not necessarily imply origin from the neural crest as postulated by Pearse or from any particular endocrine progenitor cell. Rather, the development of APUD characteristics seems to be one of several pathways of differentiation available to primitive epithelial cells [15]. Thus the term neuroendocrine has gradually replaced APUD. The neuroendocrine cells are widely distributed in normal organs and different tumors and neuroendocrine tumors have been described in virtually every organ of the body. While most of the apudomas presented with clinical symptoms related to peptide hypersecretion, many neuroendocrine tumors originating from dispersed neuroendocrine cells seem to represent subgroups of tumors arising in organs like the colon and lung [16].

DIAGNOSIS OF ECTOPIC ACTH SYNDROME

Clinical features

In the early descriptions of the syndrome, the clinical features differed from those of classic Cushing's syndrome in that it occurred predominantly in men, the duration of symptoms was less than 6 months, attributed to the malignant character of the underlying neoplasm, with body weight loss, hypertension, hyperpigmentation associated with hypokalemia and abnormal glucose tolerance. In contrast to those earlier descriptions, patients with ectopic ACTH syndrome from slower growing tumors such as carcinoids exhibit clinical features which are frequently indistinguishable from pituitary-dependent Cushing's disease. In these patients, the differential diagnosis is sometimes extremely difficult, particularly because some of them are very small and remain occult for long times before they become clinically apparent. The clinical features in the recently reported series [6, 12], including our 12 patients [9], indicate that 40–44% were in men, mean age from 30 to 50 y, duration of symptoms ranged from 1 month (small cell carcinoma of the lung) to 6 y. Cushingoid features were found in 60–100% of the patients. Weight gain paralleled centripetal obesity while 20–25% of the patients lost weight, particularly those with overt tumors. Hypertension was common (62–92%), as well as edema (55–60%), and hyperpigmentation was observed in 32–50% of the patients. Hirsutism was noticed in more than 70% of the women in two series [6, 9].

Biochemical features and baseline corticoid abnormalities

Hypokalemia was present in 71–100% of the patients and more than 80% exhibited metabolic alkalosis; both being common even in "occult" forms of ectopic ACTH syndrome. Glucose intolerance or frank diabetes mellitus was observed in 67–90% of patients [6].

Basal plasma cortisol. Basal plasma cortisol was often but not always elevated, considering the pattern of adrenal secretory bursts. In three series being reviewed, elevated fasting plasma cortisol was observed in 75–94% of the cases [6, 9 12].

Urinary 17-hydroxycorticosteroids (17-OHCS). 17-OHCS, an index of the daily cortisol secretion rate, was found to be increased in more than 95% of patients with ectopic ACTH.

Urinary "free" cortisol. Urinary "free" cortisol, thought to be a more sensitive indicator of endogenous hypercortisolism than 17-OHCS, enabled a complete separation between Cushing's syndrome, including ectopic ACTH vs normal subjects [17], presenting a 96% sensitivity, 98% specificity and an accuracy of 97% for Cushing's syndrome. In the Mayo Clinic experience baseline urinary free cortisol levels were found to be elevated in 100% of patients with ectopic ACTH [7], which was confirmed in our series [9].

Plasma ACTH. Levels (RIA) of plasma ACTH are elevated in both Cushing's disease and ectopic ACTH syndrome with some overlap between them and with a trend of higher basal levels in the ectopic syndrome. The patients with overt tumors have the highest ACTH values vs those with "occult" tumors in whom levels may be indistinguishable from that of Cushing's disease. Plasma ACTH values of 200 pg/ml or above were seen in 33–72% of patients with ectopic ACTH reviewed.

Patients with overt tumors had mean plasma cortisol and ACTH levels much higher than those observed in "occult" tumors (50–60% being thoracic carcinoids). With the more recent two-site immunoradiometric assay (IRMA) for ACTH, the biologically inactive ACTH fragments or precursors are not detected as in the RIA, giving misleadingly low values in ectopic ACTH. A ratio IRMA/RIA less than 1 is suggestive of ectopic ACTH. Alternatively, the concentrations of ACTH precursors could be determined [18] and the values in such an assay were much greater in ectopic ACTH than in Cushing's disease.

Hypothalamic–pituitary–adrenal axis in ectopic ACTH syndromes (Fig. 1)

High-dose dexamethasone suppression test. A greater than 50% drop in 17-OHCS, urinary free or total cortisol on day 2 of high-dose (8 mg) dexamethasone administration or a fall of 50% or more of 8 a.m. plasma cortisol on the morning after the second day of the glucocorticoid administration is considered to be a

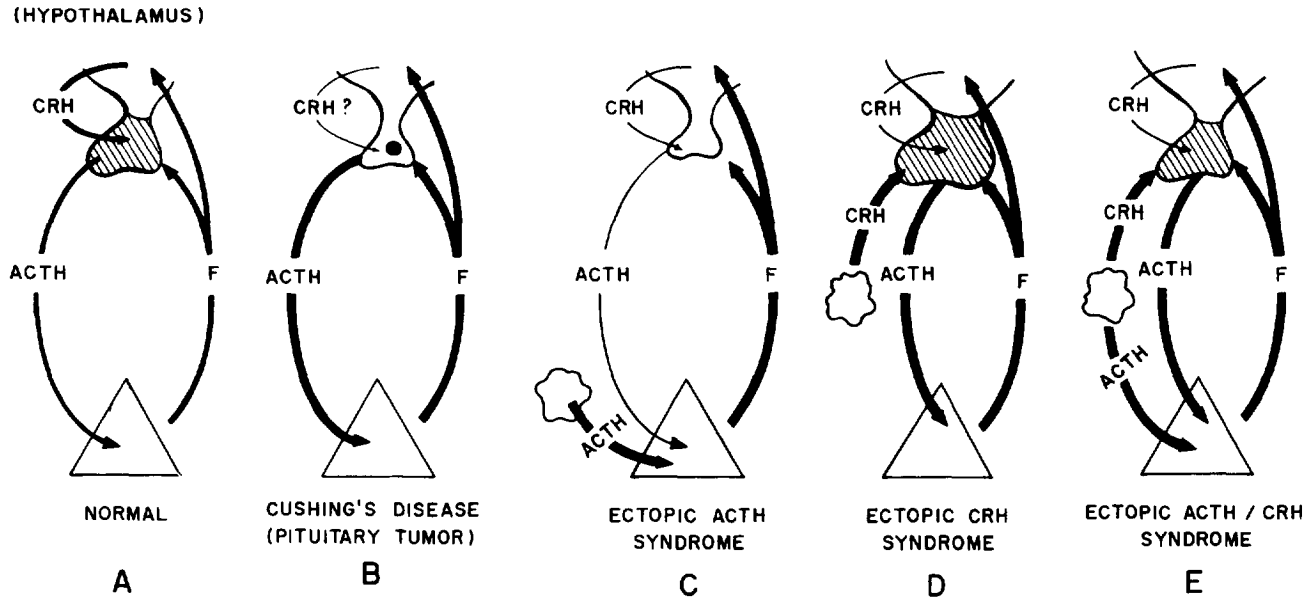

Fig. 1. Hypothalamic–pituitary–adrenal axis in normal subjects (A), Cushing's disease (B), ectopic ACTH (C), ectopic CRH (D), and ectopic ACTH–CRH syndromes (E).

positive test for pituitary disease, whereas an absence of this response suggests primary adrenal disease or ectopic ACTH secretion. In the series reviewed in Table 2 (Refs [6, 7, 9, 12, 19, 20]) it can be seen that 14–33% of the patients with ectopic ACTH suppressed urinary 17-OHCS and 12–40% decreased free or total cortisol.

Since 9–25% of patients with Cushing's disease fail to suppress urinary 17-OHCS and 12–33% of the ectopic ACTH patients in the series reviewed in Table 2 exhibited a greater than a 50% fall in urinary cortisol after dexamethasone we could have been misled in the diagnosis utilizing urinary suppression data after dexamethasone.

To circumvent the problem of urine collection, the plasma cortisol response to the standard 2-day dexamethasone test was employed. A 50% fall in plasma cortisol was observed from none to 22% in the series presented in Table 2. However, greater diagnostic accuracy was achieved by measuring urinary cortisol than plasma cortisol on the morning after the last dexamethasone dose.

As an alternative to the 2-day high-dose dexamethasone test, an 8 mg overnight test was developed, a fall of morning cortisol levels to less than 50% of baseline values strongly suggest pituitary-dependent Cushing's disease; a negative response (lack of suppression) is seen in adrenal or ectopic tumor (Table 2). The comparison of the overnight high-dose dexamethasone suppression test with the 2-day test [19, 21] indicated that there was greater sensitivity and accuracy with the overnight test.

Table 2. High-dose dexamethasone suppression test in ectopic ACTH syndrome (values expressed in percentage of the number of patients presenting ⩾50% suppression from basal levels)

	2-day high dose dexamethasone			Overnight high-dose dexamethasone
	Urinary 17-OHCS	Urinary free cortisol	8 a.m. plasma cortisol	8 a.m. plasma cortisol
Jet *et al.* [12]				
25 patients	—	25	—	—
Howlett *et al.* [6]				
16 patients	33	—	11	—
Tyrell *et al.* [19]				
7 patients	20	—	0	0
Carpenter [7]				
21 patients	14	12	—	10
Flack *et al.* [20]				
10 patients	20	40	—	—
Wajchenberg *et al.* [9]	25	33*	22	—
Cushing's disease	77	90	79	92

*Urinary total cortisol.

Metyrapone test. Using the criterion of a 100% or more increase in urinary 17-OHCS, a positive metyrapone test (750 mg × 6) was found in 33% of patients with ectopic ACTH in the combined data from several series [6, 9, 12, 17]. It is interesting to note that 63% of patients with "occult neoplasm" (primarily thoracic carcinoids) exhibited a positive response to metyrapone stimulation. Measuring plasma 11-deoxycortisol and cortisol after metyrapone, a normal response would be an increase in 11-deoxycortisol of 4.3 times basal levels, along with a plasma cortisol of less than 7 μg/dl. While 92% of patients with Cushing's disease had a normal or hyperactive response to metyrapone only 25% of our patients [9] presented such a response. The low specificity of the test in comparison to the high-dose dexamethasone suppression for the differential diagnosis of Cushing's syndrome has led investigators to recommend that this test be abandoned.

As for dexamethasone, an overnight metyrapone test was introduced (30 mg/kg of body weight, or 2–3 g) giving the drug between 11 and 12 p.m. and measuring cortisol and 11-deoxycortisol at 8 a.m. the day of the test and 8 a.m. next morning. A rise in 11-deoxycortisol greater than 5-fold with a simultaneous fall of cortisol to less than 8 μg/dl is considered as a positive response. In the Mayo Clinic experience, 97% of the patients with Cushing's disease had a positive response while none of the subjects with adrenal tumors and 12% with ectopic ACTH had a positive test (all bronchial carcinoids) [7]. According to their results, 11-deoxycortisol levels after acute administration of metyrapone has a diagnostic accuracy, sensitivity and specificity similar to that achieved by 2-day high dose dexamethasone testing or 8 mg given overnight.

Ovine corticotropin-releasing hormone (oCRH) stimulation. A positive response to oCRH was defined as an increase in ACTH value of 50% or greater or an increase in cortisol level of 20% or more from baseline. 86% of Cushing's disease patients had an ACTH response to oCRH while only 9.5% with ectopic ACTH had a normal response. The cortisol responsiveness was positive in 91% of patients with pituitary-dependent Cushing's syndrome and only 5% of ectopic ACTH responded (usually bronchial carcinoid) with a cortisol increment. Thus, cortisol response to oCRH has greater sensitivity, specificity and accuracy than measurement of ACTH. The oCRH had the same power in distinguishing pituitary from ectopic causes of Cushing's syndrome than the classic 2-day high-dose dexamethasone suppression test. In the patient with an ectopic ACTH tumor who responds to oCRH with increased ACTH levels in peripheral blood, petrosal sinus sampling during the oCRH test should help elucidate if the pituitary is the source of ACTH elevation. Ratios of ACTH in inferior petrosal sinus/peripheral blood are not altered substantially after oCRH, indicating that ACTH production occurred outside the pituitary gland. These results suggest that the ectopic tumors which are sensitive to oCRH have receptors to CRH. Since many of them also appear to respond to changes in glucocorticoids it can be assumed that the tumor has receptors to CRH and/or glucocorticoids as well as the necessary post-receptor machinery.

Inferior petrosal sinus ACTH sampling before and after oCRH stimulation. ACTH concentrations are much greater in the inferior petrosal sinus (IPS) than at peripheral sites in patients with Cushing's disease while in ectopic ACTH syndrome there should not be a significant difference between central and peripheral ACTH concentrations.

Bilateral simultaneous IPS ACTH sampling after oCRH stimulation is probably the best test for diagnosis of Cushing's disease vs ectopic ACTH syndrome, being particularly useful in differentiating the "occult" form. A maximal IPS/peripheral (IPS/P) ACTH ratio $\geqslant 2$ during basal sampling and $\geqslant 3.0$ at 3 or 5 min after oCRH indicates a pituitary source of ACTH hypersecretion [22] whereas a ratio of 1.5 or less would support an ectopic cause [23] (Table 3). In 2 patients from our series we used 10 μg of desmopressin (DDAVP) a synthetic analog of arginine-vasopressin whose ACTH-releasing ability is comparable of that of 100 μg of oCRH [24]. The maximal dominant basal IPS/P ACTH was greater than 2 in 95% of Cushing's disease patients studied by Oldfield *et al.* [20] and Findling *et al.* [23] and 73% of our patients. False-positive IPS/P–ACTH ratios of greater than 2 have been detected in the ectopic ACTH syndrome [25] when sampling occurred only in the basal state. After oCRH administration, the dominant IPS/P–ACTH ratio was >3 in 226/227 patients with Cushing's disease (pooled data as per Table 3). In all patients with ectopic ACTH syndrome, the basal IPS/P–ACTH ratio of less than 2 failed to increase or increased very little (always less than 3) after oCRH or DDAVP administration.

TUMOR MARKERS IN ECTOPIC ACTH SYNDROME

62% of the patients studied by Howlett *et al.* [6] and 72% of the patients in our series [9] exhibited elevated values of tumor markers in addition to ACTH.

ACTH precursors

The presence of high molecular weight ACTH precursors in the circulation of patients with Cushing's syndrome is suggestive of an extra-pituitary tumor. Using an IRMA based on 2 monoclonal antibodies which recognize POMC and/or ProACTH, Stewart *et al.* [3] found plasma levels of ACTH precursors markedly elevated in all 15 patients with ectopic ACTH in comparison with 20 patients with Cushing's disease. The very high ratio of ACTH precursors/ACTH is probably the best circulating marker for

Table 3. Simultaneous bilateral IPS-ACTH sampling for the diagnosis of Cushing's disease vs ectopic ACTH syndrome

	Basal sampling (dominant side)			Sampling after oCRH (or DDAVP) (3–5 min sampling (dominant side))			
	IPS/P*	Range	No. of patients	IPS/P	Range	No. of patients	References
Cushing's Disease	≥2	0.9–130	205/215	≥3	3.1–650	203203	Oldfield *et al.*, 1991 [8]
		1.1–92.5	18/20 (95%)		2.4–315	17/18 (94%)	Findling *et al.*, 1991 [23]
		0.8–14.6	11/15 (73%)		3.5–32.7	6/6 (100%)	Wajchenberg *et al.*, 1993 [9]
Ectopic ACTH	<2	0.9–1.9	2020 (100%)	<3	1.1–2.4	17/17 (100%)	Oldfield *et al.*, 1991 [8]
		1.1–1.5	9/9 (100%)		1.1–1.2	6/6 (100%)	Findling *et al.*, 1991 [23]
		1.3, 1.6	2/2 (100%)		0.8, 1.5†	2/2 (100%)	Wajchenberg *et al.*, 1993 [9]

*IPS/P–ACTH ratio.
†Desmopressin (DDAVP), 100 μg IV.

diagnosis of ectopic ACTH syndrome. However, there is the possibility that a patient with a non-pituitary tumor may over-process POMC resulting in only authentic ACTH in the circulation.

Calcitonin

This hormone was elevated in 69% of patients in Howlett's series [6] and in 44% of our patients, with bronchial carcinoids, pheochromocytomas alone or associated with medullary carcinoma of the thyroid (MEA 2A) [9].

Gut hormones

Gastrin was the most frequently elevated gut hormone in our series with very high levels found in the 2 patients with ACTH-secreting gastrinomas. Increased levels of somatostatin were found in one overt and 3/10 occult tumors reported by Howlett *et al.* [6].

Oncofetal proteins

The relationship of these proteins to embryonic tissue type and more importantly to levels of differentiation appear to determine to a large degree which oncofetal marker will be produced in greatest amount. Our only patient with elevated levels of α-fetoprotein was a 15 year old boy with an ACTH producing pancreatic islet cell tumor. CEA is the tumor marker found in highest amounts in embryonic endodermal tissues such as gastrointestinal tract, liver and pancreas, being found elevated in 4/11 (25%) of the patients in Howlett's series [6] but in none of our patients [9].

Placental markers

As for the placental marker hCG (human chorionic gonadotropin) or its β-chain (β-hCG), it has been demonstrated in 6–13% of all carcinomas [26]. We did not observe any increase in β-hCG in any of our 8 patients tested and it was elevated in only one of Howlett's subjects [6].

5-Hydroxyindolacetic acid (5-HIAA)

Urinary 5-HIAA was elevated in only one patient in Howlett's series [6] with a pancreatic endocrine tumor and in one of our patients with bronchial carcinoid and pulmonary metastases. Carcinoid tumors of the respiratory and upper gastrointestinal tract rarely produce elevated 5-HIAA or serotonin levels and are not imaged with iodine-131-meta-iodo-benzylguanidine (MIBG) scintigraphy [23].

APUD markers

Two important markers: γ-enolase or neuron-specific enolase (NSE) and chromogranins are quite helpful in determining the neuroendocrine nature of a tumor.

NSE is elevated in patients with benign and malignant APUDOMAS, namely insulinomas, pheochromocytomas, pituitary adenomas and MCT, not only in tissues but also in serum.

Chromogranins are markers for the presence of secretory granules co-stored and co-released with peptides and amines in a variety of human endocrine tissues, both normal and neoplastic, but not by non-endocrine cells. There are 3 families of chromogranins: A, B and C. Chromogranin A is the major component of the secretory granules of neuroendocrine tumors, involved in the packaging of hormones into secretory vesicles and their release from the cells together with their resident hormones [27]. The majority of patients with endocrine pancreatic tumors, carcinoids and pheochromocytomas have been shown to have increased levels of chromogranins. Among the 3 chromogranins, the A peptide is the most suitable marker of endocrine tumors. Measurement for chromogranins has a far greater sensitivity (94%) than the other serum markers [28].

IMAGING DIAGNOSIS OF ECTOPIC ACTH SYNDROME

Once the diagnosis of ectopic ACTH is made by demonstrating ACTH-dependent hypercortisolemia in the absence of an IPS/P–ACTH gradient, a screen for biochemical markers is useful to detect possible sources of ectopic ACTH-production. Next, tumor localization studies can be done recognizing that in some patients with clinically "occult" tumors the diagnosis may be difficult to establish. In the more recent series that we have reviewed [6, 9, 12, 29], 50–69% of patients with occult ACTH secretion had tumors in the chest. All patients presented with bilaterally enlarged adrenal glands on CT scans.

Chest X-rays

78% of small cell lung carcinomas were seen on initial chest X-rays in the series we have reviewed. On the other hand, 80% of the bronchial carcinoids had normal chest X-rays, requiring at times several years before a small tumor became radiographically visible. In many of these patients only a CT scan of the chest allowed definitive diagnosis. Thymic enlargement was found in 57% of thymic carcinoids on chest X-rays. Thus, routine chest X-rays can usually detect small cell lung carcinomas with overt Cushing's syndrome and some thymic carcinoids but in the majority of cases, particularly "occult tumors", normal chest X-rays are observed.

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI)

Chest CT and MRI

CT scannning of the chest was successful in localizing all thymic carcinoids we reviewed.

As for bronchial carcinoids the lesion was demonstrated on CT in 89% of the 83 surgically-proven cases reviewed by Leinung *et al.* [30] while in only 36% was an abnormal chest roentgenogram noted. MRI of the chest has detected carcinoid tumors not seen on CT [31].

Abdomen CT, MRI and ultrasonography

In those patients where CT failed to show an ectopic tumor, all other techniques are similarly unsuccessful.

Pheochromocytomas. All cases of ACTH-secreting pheochromocytomas reviewed were detected on CT scans. On MRI, pheochromocytomas are extremely bright on T2-weighted images, with the pheochromocytomas/liver ratio being uniformly greater than 3.0 [32]. Hyperplastic adrenocortical tissue, on the other hand, remains of low signal intensity or "black". The large areas of necrosis, present in many pheochromocytomas, also had high signal intensities on T2-weighted images [32]. Both benign and malignant pheochromocytomas presented similar signal intensities on T2.

Pancreatic tumors. At present, ultrasonography and CT are the methods most frequently used to identify a pancreatic tumor. Further, hepatic metastases and extrahepatic spread can be imaged via CT which provides better definition of the tumor and surrounding structures than ultrasonography. Ultrasonography has the advantage of being able to distinguish changes in echogenicity, which can reveal a small tumor before it changes the contour of the gland, a condition required for detection by CT.

An improvement in the ultrasonographic method for detection of pancreatic tumors is endoscopic ultrasonography, which provides ultrasonic imaging of pancreatic tumors and lymph nodes through the gastrointestinal lumen.

Angiography is usually not very useful in determining the size and location of the pancreatic tumor but it is an excellent way of assessing major vascular involvement. Liver metastases from pancreatic cancer and peritoneal spread can be identified by CT if masses are larger than 2 cm in diameter. Unfortunately, one third of these tumors are too small and can only be detected by direct visualization by laparoscopy before the patient is submitted to laparotomy [33].

Pancreatic neuroendocrine tumors are slowly growing tumors and the only reliable sign of malignancy is the presence of metastases or extensive growth and invasion of adjacent organs. In Eriksson's experience [34], with patients with neuroendocrine tumors of the pancreas selective abdominal angiography was the most sensitive imaging technique (67%), followed by CT scanning (65%) and ultrasonography (56%) in detecting these tumors. However, angiography was less sensitive than either CT or ultrasonography in detecting liver metastases, the latter method yielding the highest number of positive results (57%). Magnetic resonance has no significant advantage over CT in an evaluation of pancreatic tumors.

Adrenal glands. The CT appearance of the adrenal glands in ectopic ACTH syndrome may provide an early clue to the presence of ectopic ACTH production. In pituitary-dependent Cushing's disease approximately half of the adrenal glands appear normal and half mildly hyperplastic. In ectopic ACTH the glands show mild hyperplasia in 56% and marked adrenal hyperplasia in 37% of the patients [29]. In our series of 12 patients [9], enlarged adrenals were seen in all cases. As previously indicated the enlarged hyperplastic adrenals appeared of low signal intensity on T1 and T2-weighted images [29].

Medullary carcinoma of the thyroid (MCT). Cushing's syndrome is a rare hormonally-mediated complication of MCT. The most common initial clinical presentation of sporadic MCT is a single nodule or thyroid mass, almost always being "cold" on thyroid scanning, presenting hypoechoic regions on ultrasound examination. Metastases to cervical and mediastinal lymph nodes are found in half of the patients during the

examination. Thin needle aspiration of the hypoechoic nodule and cytologic examination with immunohistochemical staining for calcitonin led to a correct diagnosis in most cases of sporadic MCT.

Pituitary imaging

The diagnostic accuracy of pituitary imaging as the basis for distinguishing Cushing's disease for ectopic ACTH is compromised by the relatively high incidence of incidental pituitary microadenomas in the normal population. In Doppman's series [35], 3 of the 19 patients with proven ectopic ACTH production had positive findings in CT and/or MRI of the pituitary. MRI provides an improvement over CT scanning (47% sensitivity) with a sensitivity of 71% after Gadolinium contrast for locating a pituitary adenoma.

Should there be conflicting biochemical tests which obscure the distinction between pituitary and ectopic ACTH production, as frequently observed in bronchial carcinoids, the demonstration of a focal defect in the pituitary gland on CT or MRI should not be taken as absolute evidence for the presence of a corticotrope adenoma. Petrosal sinus samplings may be useful with the possible exception of those tumors which produce CRH without ACTH [36].

Somatostatin Receptor Scintigraphy

Lamberts and co-workers demonstrated that external imaging of many neuroendocrine tumors such as carcinoids, islet cell tumors, medullary carcinoma of the thyroid, pheochromocytomas and small cell lung cancers, using an iodinated tyrosine-3-substituted analog of octreotide, was correlated with the presence of somatostatin receptors *in vivo*. Unrecognized metastases could be demonstrated in some cases and a positive scan may predict a good suppressive effect of octreotide on hormonal secretion by these tumors [37–39].

Recent molecular genetic studies have demonstrated the existence of at least 5 different somatostatin receptor subtypes in human tissues with different tissue distributions and which differ with regard to their binding to natural somatostatin-14, somatostatin-28 and octreotide [40]. The presence of receptors, particularly of the subtypes which display selectivity to octreotide (particularly subtypes 2 and 5) provide the molecular basis for octreotide scanning and action.

Because of some disadvantages in the use of ^{123}I-Tyr-3-octreotide, in the process of labelling with radioactive iodine and the distribution of the product, a radioactive indium (^{111}In) labeled analog of somatostatin (^{111}In-DTPA-d-Phe^1-octreotide) was introduced, being superior to the tyrosine analog for the localization of a variety of tumors [41].

ACTH-secreting pituitary tumors generally do not express somatostatin receptors and no suppression of ACTH and/or cortisol was observed in patients with Cushing's disease treated with octreotide. However, octreotide therapy has been shown to suppress ACTH and cortisol secretion in several patients with ectopic ACTH secretion from pancreatic endocrine tumors, branchial carcinoids and medullary carcinoma of the thyroid [37]. Thus, somatostatin receptor scintigraphy has a place in the differential diagnosis of Cushing's syndrome and in the localization of ACTH-secreting tumors, even if they are occult. Finally, it is possible that long-term exposure to excess circulating levels of cortisol in patients with ectopic ACTH might inhibit the expression of somatostatin receptors, as has been suggested in ACTH-secreting pituitary tumors [40]. Thus, the use of mitotane or RU 486 may up-regulate somatostatin receptors in such tumors allowing their localization [40, 42].

STUDIES OF TUMOR TISSUE IN ECTOPIC ACTH SYNDROME

ACTH content of tumor tissue

The ACTH content in tumor extracts from patients with ectopic ACTH has been reported in 3 series [2, 43, 44] including 5 of our patients. Using an antiserum against the N-terminal sequence of ACTH or intact 1–39 ACTH, values ranged from 0.001 to 64.6 μg/g wet weight of tissue. On the other hand, the immunoreactivity against the C-terminal sequences, in 2 series [43, 44], varied from 0.017 to 1.1×10^6 μg/g wet weight tissue. There was in general, a good correlation between bioactivity and the N-terminal ACTH immunoreactivity in the Orth series [43].

Altered POMC processing in ectopic ACTH syndrome

POMC, a glycopeptide of molecular weight 31 kDa, is a common precursor of ACTH, β-lipotropin (β-LPH), β-endorphin (β-END) and a series of other peptides (Fig. 2). Posttranslational processing of POMC and its cleavage into smaller peptides may differ between the pituitary gland and nonpituitary tumors [45]. POMC possesses 9 potential cleavage sites [46]; not all of these are used, and different sites may be used in different cell types which express the precursor gene, leading to a tissue-specific set of peptides. In the human pituitary, a specific prohormone convertase (PC3 endoprotease) results in POMC processing of 4 sites, giving rise to only 6 peptides: N-POMC$_{1-76}$, joining peptide (JP), ACTH, B-LPH and small amounts of γ-LPH and β-END. In non-pituitary tumors, there may be alternate POMC-processing, resulting either in large underprocessed molecules, such as ProACTH or activation of normally silent cleavage sites, suggesting the expression of PC2 endoprotease, generating fragments not commonly seen in normal or tumorous pituitary. Fragments such as corticotropin-like intermediate lobe polypeptide (CLIP) and β-MSH$_{5-22}$ may be produced by activating the β-MSH$_{5-22}$ site from γ-LPH. In non-pituitary tumors there is a high ratio of CLIP relative to the other fragment, suggesting that the CLIP site may be

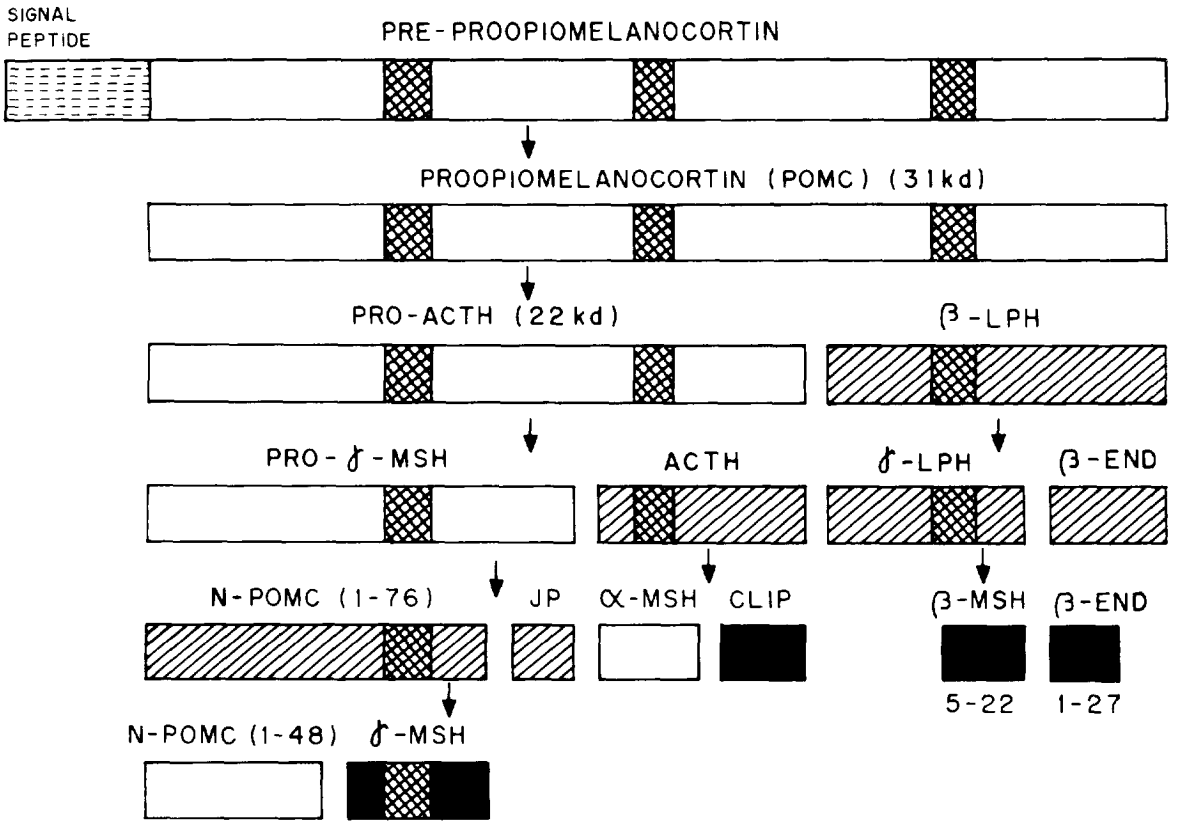

Fig. 2. Posttranslational processing of proopiomelanocortin (POMC) in normal pituitary, pituitary and ectopic ACTH-secreting tumors. Hatched areas indicate peptides more abundant in the pituitary; cross-hatched areas indicate MSH sequences and black areas represent "abnormal" fragment markers of ectopic ACTH syndrome. (Adapted from D. Vieau *et al.* [46] with permission of the publishers, and from A. C. Hale *et al.* [45] with permission of the authors and publishers).

a more sensitive site than β-MSH_{5-22} for activating an alternate mode of POMC processing [47].

On the other hand, a higher proportion of ProACTH has been demonstrated in tumor extracts from patients with ectopic ACTH than from pituitary-dependent Cushing's disease, Addison disease and Nelson's syndrome [44]. Other molecular forms present in ectopic ACTH-secreting tumors were N-terminal $POMC_{1-76}$, $ACTH_{1-39}$, β-LPH, γ-LPH and β-END. These fragments were also observed in pituitary tumors, but at much lower levels. Further, there were smaller ACTH fragments, including an $ACTH_{2-38}$ fragment (from authentic $ACTH_{1-39}$ by specific endoproteases) absent from the pituitary, and fully active in an *in vivo* assay.

Thus, the measurement of abnormal fragments such as CLIP, β-MSH, γ-MSH and a high β-LPH to γ-LPH ratio in the plasma of a patient with ACTH-dependent Cushing's syndrome may be useful in determining an ectopic origin of the ACTH.

APUD markers in ACTH-secreting tumors

The current methods for recognizing neuroendocrine tumors involve the demonstration of one or more of the following classes of markers: (1) synthesis of amines and/or peptides; (2) enzymes such as the neuron-specific enolase (γ-enolase); (3) secretory granules such as the chromogranins; (4) secretory granule peptides 7B2 and CCB; and (5) specific receptors, such as somatostatin and analog peptides. The importance of documenting that a tumor is of neuroendocrine origin lies in the observation that certain chemotherapeutic regimens worked unexpectedly well in anaplastic carcinomas if they are of neuroendocrine origin [48].

Nine patients from our series with ectopic ACTH secretion were studied by immunohistochemical methods in the primary tumor and/or metastatic lesions (Table 4). In all but one, ACTH was detected in the primary neoplastic cells and/or metastases by an immunoperoxidase method. All neoplastic tissues immunostained for neuron-specific enolase and chromogranin-A were positive which, as indicated, identified the tumors as neuroendocrine. An inverse correlation between immunohistochemical results and clinical observations is usually remarkably good. Thus, rapidly growing tumors with widespread metastases, such as small cell lung carcinomas, did not contain sufficient ACTH for cytochemical confirmation [49, 50]. It could be suggested that highly malignant endocrine tumors associated with ectopic ACTH exhibit excessive hormone secretion rates, leading to low

Table 4. Immunohistochemical evaluation of the primary tumor and/or metastases in 9 patients with ectopic ACTH syndrome studied at The Hospital das Clínicas (São Paulo, Brazil)

Patient	Primary tumor	Tissue studied	Immunoreactivity					
			ACTH	Neuron-specific enolase (γ enolase)	Chromogranin-A	Calcitonin	Gastrin	CRH
1	Thymus carcinoid	Primary tumor	+					+ +
2	Thymus carcinoid	Primary tumor	+	+ +				
		Ovarian metastasis	+	+				
		Breast metastasis	+	+				
		Lymph node metastasis		+				
		Brain metastasis		+	+ + +			
3	Thymus carcinoid	Primary tumor	+ +	+ + +	+ + +	−	−	
		Breast metastasis	+	+ + +	+ + +	−	−	
		Liver metastasis		+ + +	+ +			
		Adrenal metastasis		+	+			
4	Bronchial carcinoid	Primary tumor	+					
5	Metastatic lung carcinoid	Lung metastasis	+	+ + +	+ + +	+	−	
7	Pancreatic neuroendocrine neoplasia	Primary tumor	+	+ +	+ + +	−	−	
8	Metastatic gastrinoma	Liver metastasis	−	+ + +	+ + +	−	+	
9	Bilateral pheo	Left adrenal	+ +	+ +	+ + +	−	−	
	+ medullary	Right adrenal	+ +	+ +	+ + +	−	−	
	carcinoma thyroid	Thyroid carcinoma	−	+ +	+ + +	+ + +	−	
11	Malignant right pheo	Primary tumor	+	+ + +	+ + +	+	−	

Pheo, pheochromocytoma.

storage of peptide hormones such as the POMC-derived-ones.

"OCCULT" ECTOPIC ACTH SYNDROME

As defined by Doppman [35], "occult" ectopic ACTH syndrome is CRH- and/or ACTH-dependent hypercortisolemia of non-pituitary origin and of more than 4–6 months duration without the emergence of an obvious source. The "occult" ectopic ACTH syndrome is often clinically indistinguishable from Cushing's disease, since biochemical studies and imaging evaluation may yield inconclusive or misleading results. Failure to recognize "occult" ectopic ACTH syndrome may lead to unnecessary pituitary/adrenal surgery and has created a need for improved diagnostic procedures such as IPS ACTH sampling.

In a review of 68 patients with "occult" ectopic ACTH including 10 subjects from our series [9, 23, 51], the mean patient age was 44 y and the male/female ratio was almost 1, in contrast with the striking female preponderance in pituitary-dependent Cushing's disease. The general physical features were indistinguishable from those of Cushing's disease, however hypokalemia was present in 68% of the patients vs less than 10% in Cushing's disease. In many patients basal ACTH levels were similar to that found in Cushing's disease (ACTH/RIA less than 200 pg/ml). Intermittent hypercortisolemia with long periods of remission has been increasingly appreciated in ectopic ACTH resulting in misinterpretation of laboratory investigations.

32 of 56 patients with "occult" ectopic ACTH had bilateral adrenalectomy, either because the initial diagnosis was Cushing's disease or the source of ectopic ACTH could not be detected at the time. It is likely that many "occult" tumors might have been detected earlier using today's technology and without the need for palliative bilateral adrenalectomy.

Regarding the dynamic tests, 30% of the patients with occult ACTH-producing tumors exhibit high-dose dexamethasone suppressibility and as many as 41% of thoracic carcinoids associated with ectopic ACTH are dexamethasone-suppressible [52]. As for the metyrapone test, 50–63% of the patients had a positive response. Together with the high-dose dexamethasone suppressibility in a high percentage of cases, these findings are highly suggestive of pituitary-dependent Cushing's syndrome, probably reflecting the function of glucocorticoid receptors in these tumors [30].

IPS ACTH sampling was able to distinguish patients with "occult" ectopic ACTH syndrome from those with pituitary-dependent Cushing's disease [13, 23].

Ectopic ACTH tumors due to carcinoids can be confused with pituitary Cushing's disease because of positive dexamethasone suppression and metyrapone tests [52] and even oCRH stimulation. The mechanism of tumor response to these challenges in unclear most probably due to tumor response to changes in

circulating glucocorticoid levels but also to CRH [53]. It has been demonstrated that some of the tumors secrete CRH with or without ACTH. However, it is unlikely that tumors secreting both CRH and ACTH could exhibit CRH stimulation of pituitary ACTH, since the hypercortisolemia resulting from tumor-secreted ACTH would block CRH action on the pituitary [54]. Then, it could be postulated that the tumors that respond to dexamethasone, metyrapone and CRH are those which produce CRH without associated ACTH.

Regarding the types of tumors producing occult ectopic ACTH, in our review of 68 cases, 60% were thoracic carcinoids (3/4 being bronchial carcinoids), followed by SCLC (10%), pancreatic neuroendocrine neoplasia and miscellaneous tumors in decreasing frequency. The source of ectopic ACTH was not detected in 12% of the "occult" tumors. Once the diagnosis of the ectopic ACTH syndrome is established a screen for tumor markers to detect possible sources of ectopic ACTH should be performed. Since more than 90% of the patients with occult ectopic ACTH syndrome harbor neuroendocrine tumors, elevated circulating levels of neuron-specific enolase and/or chromogranins should be present. If biochemical screening provides no clue for the source of ectopic ACTH, CT/MRI/somatostatin scintigraphy of the chest and abdomen should be performed. When the initial screening fails to detect the source of the ectopic ACTH production, medical suppression of adrenal cortisol hypersecretion may be tried along with periodic screening with CT/MRI/scintigraphy. Even if failure of medical suppression leads to bilateral adrenalectomy, surveillance must continue so that potentially malignant carcinoid and neuroendocrine islet cell tumors can be detected at the earliest possible time.

ECTOPIC CRH SYNDROME

Six cases of "isolated" ectopic CRH have been reported (2 metastatic prostatic carcinomas, 2 metastatic medullary carcinoma of the thyroid, 1 intrasellar medullary carcinoma of the thyroid, 1 intraseller gangliocytoma and 1 metastatic small cell carcinoma with unknown primary tumor). In all cases the tumor and/or metastases were immunostained positive for CRH but not for ACTH. The ectopically produced CRH stimulated excessive secretion of pituitary ACTH, as shown in Fig. 1. The pituitary glands were examined in 5 patients and revealed corticotrope hyperplasia in 4 and normal in the 5th case. All 5 cases demonstrated ACTH in their corticotropes by immunostaining. Peripheral levels of CRH when measured (3 patients) were elevated. In most cases the diagnosis of ectopic CRH was made either postmortem or postsurgery.

The circulating levels of ACTH and cortisol were in the range usually found in patients with "occult" tumors or with Cushing's disease. In 4 patients in whom high-dose dexamethasone suppressibility had been tested, none showed a positive response.

Although these patients are categorized as selectively secreting CRH, it is possible that their tumors co-secreted ACTH as well and just negative staining for ACTH in the tumor or metastases may be related to reduced storage of ACTH due to high secretion rates, as previously indicated. Positive histochemical evidence of pituitary ACTH hypersecretory activity and hyperplastic appearance of the corticotrope cells in nearly all of the patients in whom pituitary tissue was available suggests that tumor ACTH might have been produced in relatively small amounts, not blocking the action of CRH on the pituitary. Thus, the major production of ACTH would be of pituitary origin.

ECTOPIC CRH–ACTH

There have been 13 cases of combined ectopic CRH–ACTH described in the literature and one from our series [55]. This series includes 3 patients with metastatic lung small cell carcinoma, 3 cases of bronchial carcinoids, 2 thymic carcinoids, 1 pancreatic carcinoma, 1 medullary carcinoma of the thyroid, 1 pheochromocytoma, 1 MEN I + paraganglioma and 1 nephroblastoma. In these 13 patients the tumor was considered to produce both peptides, based on immunocytochemical studies of the tumor and/or metastatic tissue and by the finding of a CRH and ACTH concentration gradient across the tumor bed in 2 cases [22, 56]. Six of 11 patients (54%) in whom circulating ACTH was measured by RIA had values <210 pg/ml; 4 of these being thoracic carcinoids. On the other hand, our patient with thymic carcinoid had the highest ACTH and cortisol values in the series [55].

Plasma cortisol was above 28 μg/dl in 9/11 (82%) of the patients studied with combined ectopic CRH–ACTH. The plasma CRH was above the normal range in all 7 patients in which it was measured, except in a case of a thymic carcinoid reported by Raux Demay *et al.* [57] which had normal circulating hCRH. In this case, the tumor tissue was demonstrated to contain POMCmRNA and POMC-related peptides such as $ACTH_{1-39}$ in addition to hCRH. In the absence of elevated circulating CRH, tumor CRH could have exercised a paracrine function, stimulating the tumor cells to produce POMC peptides [58].

As for dynamic tests, 4 of the 7 patients studied (57%) had a positive dexamethasone suppression while all 6 patients in whom a metyrapone test was done showed a positive response. Four of the 5 patients in whom dexamethasone suppression and metyrapone stimulation were performed, the responses were characteristic of pituitary-dependent Cushing's disease.

The finding of corticotrope hyperplasia in the case of nephroblastoma [59] and another patient with bronchial carcinoid [25] along with pituitary

immunoreactivity to ACTH (both patients responded to dexamethasone and metyrapone) suggest that the major production of ACTH was by pituitary stimulation from the tumor CRH, with the role of tumor ACTH being a minor one.

In conclusion, in patients with ectopic CRH–ACTH production by extrapituitary neoplasms there is a range of clinical presentations from classical ectopic ACTH-secreting tumors to biochemical patterns found in pituitary-dependent Cushing's disease.

REFERENCES

1. Odell W. D., Wolfsen A. R., Yoshimoto Y., Weitzman R. and Hirose F.: Ectopic peptide synthesis—a universal concomitant of neoplasia. *Trans. Assoc. Am. Phys.* **90** (1977) 204–227.
2. Gewirtz G. and Yalow R. S.: Ectopic ACTH production in carcinoma of the lung. *J. Clin. Invest.* **53** (1974) 1022–1032.
3. Stewart P. M., Gibson S., Crosby S. R., Penn R., Holder R., Ferry D., Taicher N., Phillips P., London D. R. and White A.: ACTH precursors characterize the ectopic ACTH syndrome. *Clin. Endocr. (Oxf.)* **40** (1994) 199–204.
4. White A. and Clark A. J. L.: The cellular and molecular basis of the ectopic ACTH syndrome. *Clin. Endocr. (Oxf.)* **39** (1993) 131–141.
5. Clark A. J. L., Stewart M. F., Lavender P. M., Farrell W., Crosby S. R., Rees L. H. and White A.: Defective glucocorticoid regulation of proopiomelanocortin gene expression and peptide secretion in a small cell lung cancer cell line. *J. Clin. Endocr. Metab.* **70** (1990) 485–490.
6. Howlett T. A., Drury P. L., Perry L., Doniach I., Rees L. H. and Besser G. M.: Diagnosis and management of ACTH-dependent Cushing's Syndrome: comparison of the features in ectopic and pituitary ACTH production. *Clin. Endocr. (Oxf.)* **24** (1986) 699–713.
7. Carpenter P. C.: Diagnostic evaluation of Cushing's syndrome. *Endocr. Metab. Clin. North Am.* **17** (1988) 455–472.
8. Oldfield E. H., Doppman J. L., Nieman L. K., Chrousos G. P., Miller D. L., Katz D. A., Cutler G. B. Jr. and Loriaux D. L.: Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. *New Engl. J. Med.* **325** (1991) 897–905.
9. Wajchenberg B. L., Mendonça B. B., Liberman B., Pereira M. A. A., Carneiro P. C., Wakamatsu A. and Kirschner M. A.: The changing picture of ectopic ACTH syndrome. *Arch. Intern. Med.* Submitted.
10. Liddle G. W., Nicholson W. E., Island D. P., Orth D. N., Abe K. and Lowder S. C.: Clinical and laboratory studies of ectopic tumoral syndromes. *Recent Prog. Horm. Res.* **25** (1969) 283–324.
11. Imura H., Nakai Y., Nakao K., Oki S., Matsukura S., Hirata Y., Fukase M., Hattori M., Yoshmini H. and Sueoka S.: Functioning tumors with special reference to ectopic hormone producing tumors. *Prot. Nucl. Acid Enzyme* **23** (1978) 641–656.
12. Jex R. K., Van Heerden J. A., Carpenter P. C. and Grant C. S.: Ectopic ACTH syndrome. Diagnostic and therapeutic aspects. *Am. J. Surg.* **149** (1985) 276–282.
13. Doppman J. L., Nieman L., Miller D. L., Pass H. I., Chang R., Cutler G. B., Schaaf M., Chrousos G. P., Norton J. A., Ziessman H. A., Oldfield E. H. and Louriaux D. L.: Ectopic adrenocorticotropic hormone syndrome: localization studies in 28 patients. *Radiology* **172** (1989) 115–124.
14. Pearse A. G. E.: The APUD concept and hormone production. *Clin. Endocr. Metab.* **9** (1980) 211–222.
15. Baylin S. B. and Mendelsohn G.: Ectopic (inappropriate) hormone production by tumors: mechanisms involved and the biological and clinical implications. *Endocr. Rev.* **1** (1980) 45–77.
16. Lamberts S. W. J., Krenning E. P. and Reubi J-C.: The role of somatostatin and its analogues in the diagnosis and treatment of tumors. *Endocr. Rev.* **12** (1991) 450–482.
17. Crapo L.: Cushing's syndrome: a review of diagnostic tests. *Metabolism* **28** (1979) 955–977.
18. Crosby S. R., Stewart M. F., Ratcliffe J. G. and White A.: Direct measurement of the precursors of adrenocorticotropin in human plasma by two-site immunoradiometric assay. *J. Clin. Endocr. Metab.* **67** (1988) 1272–1277.
19. Tyrrell J. B., Findling J. W., Aron D. C., Fitzgerald P. A. and Forsham P. H.: An overnight high dose dexamethasone suppression test for rapid differential diagnosis of Cushing's syndrome. *Ann. Intern. Med* **104** (1986) 180–186.
20. Flack M. R., Oldfield E. H., Cutler G. B. Jr., Zweig M. H., Halley J. D., Chrousos G. P., Loriaux D. L. and Nieman L. K.: Urine free cortisol in the high-dose dexamethasone suppression test for the differential diagnosis of the Cushing's syndrome. *Ann. Intern. Med.* **116** (1992) 211–217.
21. Bruno O. D., Rossi M. A., Contreras L. N., Gomez R. M., Galparsoro G., Cazado E., Kral M., Leber B. and Arias D.: Nocturnal high-dose dexamethasone suppression test in the aetiological diagnosis of Cushing's syndrome. *Acta Endocr. (Copenh.)* **109** (1985) 158–162.
22. Schteingart D. E., Lloyd R. V., Akil H., Chandler W. F., Ibarra-Perez G., Rosen S. G. and Ogltree R.: Cushing's syndrome secondary to ectopic corticotropin-releasing hormone-adrenocorticotropin secretion. *J. Clin. Endocr. Metab.* **63** (1986) 770–775.
23. Findling J. W., Kehoe M. E., Shaker J. L. and Raff H.: Routine inferior petrosal sinus sampling in the differential diagnosis of adrenocorticotropin (ACTH)-dependent Cushing's syndrome; early recognition of occult ectopic ACTH syndrome. *J. Clin. Endocr. Metab.* **73** (1991) 408–413.
24. Malerbi D. A., Mendonça B. B., Liberman B., Toledo S. P. A., Corradini M. C. M., Cunha-Neto M. B., Fragoso M. C. B. V. and Wajchenberg B. L.: The desmopressin stimulation test in the differential diagnosis of Cushing's syndrome. *Clin Endocr. (Oxf.)* **38** (1993) 463–472.
25. Case records of the Massachusetts General Hospital weekly Clinico-pathological exercises: Case 52. *New Engl. J. Med.* **317** (1987) 1648–1658.
26. Odell W. D. and Appleton W. S.: Humoral manifestations of cancer. In *Williams Textbook of Endocrinology, 8th edition* (Edited by J. D. Wilson and D. W. Foster). WB Saunders Co., PA (1992) 1599–1617.
27. O'Connor D. T. and Deftos L. J.: Secretion of chromogranin A by peptide-producing endocrine neoplasms. *New Engl. J. Med.* **313** (1986) 1145–1151.
28. Eriksson B., Arnberg H., Oberg K., Hellman V., Lundqvist G., Wernstedt C. and Willander E.: A polyclonal antiserum against chromogranin A and B—a new sensitive marker for neuroendocrine tumors. *Acta Endocr. (Copenh.)* **122** (1990) 145–155.
29. Doppman J. L., Nieman L., Miller D. L., Pass H. I., Chang R., Cutler G. B., Schaaf M., Chrousos G. P., Norton J. A., Ziessman H. A., Oldfield E. H. and Loriaux D. L.: Ectopic adrenocorticotropic hormone syndrome: localization studies in 28 patients. *Radiology* 172 (1989) 115–124.
30. Leinung M. C., Young W. F. Jr., Whitaker M. D., Scheithauer B. W., Trastek V. F. and Kvols L. K.: Diagnosis of corticotropin-producing bronchial carcinoid tumors causing Cushing's syndrome. *Mayo Clin. Proc.* **65** (1990) 1315–1321.
31. Doppman J. L., Pass H. I., Nieman L. K., Findling J. W., Dwyer A. J., Fernstein I. M., Ling A., Travis W. D., Cutler G. B. Jr., Chrousos G. P. and Loriaux D. L.: Detection of ACTH-producing bronchial carcinoid tumors: MR imaging vs CT. *AJR* **156** (1991) 39–43.
32. Doppman J. L., Reinig J. W., Dwyer A. J., Frank J. P., Norton J., Loriaux D. L. and Keiser H.: Differentiation of adrenal masses by magnetic resonance imaging. *Surgery* **102** (1987) 1018–1026.
33. Warshaw A. L. and Del Castillo C. F.: Pancreatic carcinoma. *New Engl. J. Med.* **326** (1992) 455–465.
34. Eriksson B., Arnberg H., Lindgren P. G., Lorelius L. E., Magnusson A., Lundqvist G., Skogseid B., Wide L., Wilander E. and Oberg K.: Neuroendocrine pancreatic tumors: clinical presentation, biochemical and histopathological findings in 84 patients. *J. Intern. Med.* **228** (1990) 103–113.
35. Doppman J. L.: The search for occult ectopic ACTH-producing tumors. *Endocrinologist* **2** (1992) 41–46.
36. Schteingart D. E.: Ectopic secretion of peptides of the proopiomelanocortin family. *Endocr. Metab. Clin. North Am.* **20** (1991) 453–471.

37. Lamberts S. W. J., Krenning E. P. and Reubi J-C.: The role of somatostatin and its analogues in the diagnosis and treatment of tumors. *Endocr. Rev.* **12** (1991) 450–482.
38. Lamberts S. W. J., Hofland L. J., van Koetsveld P. M., Reubi J-C., Bruining H. A., Bakker W. H. and Krenning E. P.: Parallel *in vivo* and *in vitro* detection of functional somatostatin receptors in human endocrine pancreatic tumors. Consequences with regard to diagnosis, localization and therapy. *J. Clin. Endocr. Metab.* **71** (1990) 566–574.
39. Lamberts S. W. J., Bakker W. H., Reubi J-C. and Krenning E. P.: Somatostatin-receptor imaging in the localization of endocrine tumors. *New Engl. J. Med.* **323** (1990) 1246–1249.
40. Lamberts S. W. J., deHerder W. E., Krenning E. P. and Reubi J-C.: A role of (labeled) somatostatin analogs in the differential diagnosis and treatment of Cushing's syndrome. *J. Clin. Endocr. Metab.* **78** (1994) 17–19.
41. Krenning E. P., Kwekkeboom D. J., Bakker W. H., Kooij P. P., Oei H. Y., Van Hagen M., Postema P. T., de Jong M. and Reubi J-C.: Somatostatin receptor scintigraphy with (^{111}In-DTPA-d-Phe^1) and (^{123}ITyr-3) octreotide: the Rotterdam experience with more than 1000 patients. *Eur. J. Nucl. Med.* **20** (1993) 716–731.
42. Philipponneau M., Nocaudie M., Epelbaum J., de Keyser Y., Lalau J. D., Marchandise X., Bertagna X.: Somatostatin analogs for the localization and preoperative treatment of an adrenocorticotropin-secreting bronchial carcinoid tumor. *J. Clin. Endocr. Metab.* **78** (1994) 20–24..
43. Orth D. N., Nicholson W. E., Mitchell W. M., Island D. P., Liddle G. W.: Biologic and immunologic characterization and physical separation of ACTH and ACTH fragments in ectopic ACTH syndrome. *J. Clin. Invest.* **58** (1973) 1757–1769.
44. Hale A. C., Besser G. M. and Rees L. H.: Characterization of pro-opiomelanocortin-derived peptides in pituitary and ectopic adrenocorticotropin-secreting tumors. *J. Endocr.* **108** (1986) 49–56.
45. Vieau D., Massias J-F., Girard F., Luton J-P., Bertagna X.: Corticotrophin-like intermediary lobe peptide as a marker of alternative pro-opiomelanocortin processing in ACTH-producing non-pituitary tumors. *Clin. Endocr. (Oxf.)* **31** (1989) 691–700.
46. Whitfeld P., Seeburg P. and Shine J.: The human proopiomelanocortin gene: organization, sequence and interspersion with repetitive DNA. *DNA* **1** (1982) 133–134.
47. de Keyser Y., Bertagna X., Lenne F., Girard F., Luton J-P and Kahn A.: Altered proopiomelanocortin gene expression in adrenocorticotropin-producing nonpituitary tumors. *J. Clin. Invest.* **76** (1985) 1892–1898.
48. Moertel C. G., Kvols L. K., O'Connell M. J. and Rubin J.: Treatment of neuroendocrine carcinomas with combined etoposide and cisplatin: evidence of major therapeutic activity in the anaplastic variants of these neoplasms. *Cancer* **68** (1991) 227–232.
49. Coates P. J. H., Doniach I., Howlett T. A., Rees L. H., Besser G. M.: An immunocytochemical study of 18 tumours causing ectopic Cushing's syndrome. *J. Clin. Path.* **39** (1986) 955–960.
50. Heitz P. U., Kloppel G., Polak J. M. and Staub J. J.: Ectopic hormone production by endocrine tumors: localization of hormones at the cellular level by immunocytochemistry. *Cancer* **48** (1981) 2029–2037.
51. Findling J. W. and Tyrrell J. B.: Occult ectopic secretion of corticotropin. *Arch Intern. Med.* **146** (1986) 929–933.
52. Pass H. I., Doppman J. L., Nieman L., Stovroff M., Vetto J., North J. A., Travis W., Chrousos G. P., Oldfield E. H. and Cutler G. B. Jr.: Management of the ectopic ACTH syndrome due to thoracic carcinoids. *Ann. Thorac. Surg.* **50** (1990) 52–57.
53. Malchoff D., Orth D. N., Abboud C., Carney J. A., Pairolero P. C. and Carey R. M.: Ectopic ACTH syndrome caused by a bronchial carcinoid tumor responsive to dexamethasone, metyrapone and corticotropin-releasing factor. *Am. J. Med.* **84** (1988) 760–764.
54. Orth D. N.: Corticotropin-releasing hormone in humans. *Endocr. Rev.* **13** (1992) 164–191.
55. Wajchenberg B. L., Mendonça B. B., Liberman B., Pereira M. A. A., Carneiro P. C., Wakamatsu A. and Kirschner M. A.: Ectopic ACTH syndrome. *Endocr. Rev.* **15** (1994) 752–787.
56. Jessop D. S., Cunnah D., Millar J. G. P., Neville E., Coates P., Doniach I., Besser G. M. and Rees L. H.: A pheochromocytoma presenting with Cushing's syndrome associated with increased concentrations of circulating corticotrophin-releasing factor. *J. Endocr.* **113** (1987) 133–138.
57. Raux Demay M. C., Proeschel M. F., de Keyser Y., Bertagna X., Luton J-P. and Girard F.: Characterization of human corticotrophin-releasing hormone and pro-opiomelanocortin-related peptides in a thymic carcinoid tumor responsible for Cushing's syndrome. *Clin. Endocr. (Oxf.)* **29** (1988) 649–657.
58. Carey R. M., Varma S. K., Drake C. R. Jr., Thorner M. O., Kovacs K., Rivier J. and Vale W.: Ectopic secretion of corticotropin-releasing factor as cause of Cushing's syndrome. A clinical, morphological and biochemical study. *New Engl. J. Med.* **311** (1984) 13–20.
59. Hashimoto K., Takahara J., Ogawa N., Yunoki S., Ofuji T., Arata A., Kanda S. and Terada K.: Adrenocorticotropin, B-lipotropin, B-endorphin, and corticotropin-producing nephroblastoma. *J. Clin. Endocr. Metab.* **50** (1980) 461–465.