

Secretion of neuron-specific enolase, prolactin, growth hormone, luteinising hormone and follicle stimulating hormone by "functionless" and endocrine-active pituitary tumours in vitro

NF LAWTON, AJ EVANS,* JD PICKARD†, S PERRY,† B DAVIES‡

From the Wessex Neurological Centre and Departments of Chemical Pathology,* Surgery† and Neuropathology,‡ Southampton University Hospitals, Southampton, UK

SUMMARY Secretion of the neuroendocrine marker neuron-specific enolase by 24 pituitary tumours was measured in maintenance tissue culture. Eleven endocrine-active and 13 "functionless" tumours were defined by measurement of prolactin, growth hormone, luteinising hormone (LH) and follicle stimulating hormone (FSH) secretion rates *in vitro* and the corresponding plasma hormone levels. Measurement of prolactin secretion provided a clear distinction between true prolactinomas and "functionless" tumours causing hyperprolactinaemia by stalk compression (pseudoprolactinomas). A previous report of LH and/or FSH secretion by the majority of "functionless" tumours was confirmed, but plasma levels of LH and FSH were usually normal. It is argued that LH and FSH are not the major hormones secreted by "functionless" tumours. A high production rate of neuron-specific enolase appears to be characteristic of the cell type from which most "functionless" tumours derive.

Endocrine-active pituitary tumours secrete known hormones in excess, whereas "functionless" tumours are endocrinologically silent. Secretion by "functionless" tumours is, however, predictable because electron microscopy shows the presence of cytoplasmic secretory granules similar to granules containing known hormones in endocrine-active tumours.¹ Although the putative hormone secreted by "functionless" tumours is unknown the association of a tumour marker might throw further light on the cell type from which these tumours arise.

Neuron-specific enolase (NSE) is one candidate for such a biochemical marker. As its name implies, NSE is the $\gamma\gamma$ isomer of a glycolytic enzyme present in neurons and their axons, whilst glial cells contain the $\alpha\alpha$ isomer.² Recent studies have shown that NSE is not confined to neurons and is widely distributed in neuroendocrine tissues including the APUD (amine precursor uptake and decarboxylation) cells of the gut,³ pancreas,^{3,4} thyroid,⁴ lung⁵ and adrenal glands.⁴ NSE is therefore a cell marker for the diffuse

neuroendocrine system⁴ and is produced by a variety of neuroendocrine tumours.⁶

Marangos *et al*⁷ reported the presence of NSE in the normal anterior pituitary of man and monkey but did not comment on its distribution according to cell type. Van Noorden *et al*⁸ subsequently showed by immuno-cytochemical staining that NSE is present in all the major hormone producing cells of the normal rat and human pituitary and in tumour cells of adenomas producing growth hormone (GH), prolactin or ACTH. In the same study NSE was demonstrated in the cells of 10 "functionless" human adenomas. These findings were qualitative and it was not possible to predict the production rate of NSE by different cell types. Quantitative studies of NSE production by pituitary tumours have been confined to measurements in CSF. Royds *et al*⁹ found high CSF concentrations of enolase in nine of 13 patients with pituitary adenomas but subsequently showed that non-neuronal enolase was more frequently increased than NSE.¹⁰ The tumour type in patients with high CSF enolase levels was not reported.

We have investigated the production rate of NSE by pituitary tumours of a particular cell type by assaying NSE in the supernatant from maintenance tissue cultures of 24 adenomas. Eleven endocrine active tumours secreting growth hormone (GH)

Address for reprint requests: NF Lawton, Wessex Neurological Centre, Southampton General Hospital, Southampton SO9 4XY, UK.

Received 18 May 1985 and in revised form 8 August 1985. Accepted 25 August 1985

and/or prolactin and 13 "functionless" tumours were defined by plasma hormone levels in conjunction with measurements of hormone secretion rate *in vitro*.

The prolactin secretion rate was measured in every case in order to distinguish true prolactinomas from "functionless" tumours associated with hyperprolactinaemia, and the GH secretion rate to exclude contamination of tumour tissue by normal pituitary. The 13 tumours ultimately classified as "functionless" were chromophobe adenomas which failed to secrete prolactin or growth hormone *in vitro* despite the frequent finding of modest hyperprolactinaemia *in vivo*.

Luteinising hormone (LH) and follicle stimulating hormone (FSH) secretion was measured in this study because of conflicting evidence for the frequency with which "functionless" tumours secrete gonadotrophins. FSH and/or LH has been detected by immunoperoxidase staining in only 3.5% of pituitary adenomas in two large series.^{11,12} Mashiter *et al*,¹³ however, reported FSH and LH secretion by nine of ten apparently functionless tumours in tissue culture and suggested that gonadotrophin secretion by chromophobe tumours is more common than previously supposed.

Patients

The 24 patients in this study underwent transsphenoidal hypophysectomy for a histologically proven pituitary ade-

noma. Great care was taken at operation to distinguish adenoma from normal pituitary tissue. In patients with visual loss, removal of the adenoma and its suprasellar extension was as complete as possible with descent of the ballooned diaphragma sellae into the fossa. The compressed normal pituitary gland was not removed intentionally if it was clearly separate from adenoma. In patients with intrasellar adenomas a selective but microscopically complete removal of the adenoma was performed together with a thin cuff of adjacent normal pituitary gland. The table shows the age, sex, clinical presentation, preoperative plasma hormone levels and the tumour type in each case. Three of the five patients with prolactinomas presented with chiasmal compression only, and the two patients with amenorrhoea and infertility had intrasellar tumours. The diagnosis of acromegaly in six patients was confirmed by failure of plasma GH to suppress below 2 mU/l during a standard oral glucose tolerance test. Two patients with acromegaly also had hyperprolactinaemia and are shown as mixed tumours. Twelve patients with "functionless" tumours who presented with chiasmal compression had no manifest endocrine disturbance. One patient in this category (Patient 24) had long standing hypogonadism, a eunuchoid habitus and a plasma testosterone of 1.1 nM/l (normal 10–30 nM/l). A pituitary tumour was diagnosed on routine skull radiography after a minor head injury and the CT scan showed a small suprasellar extension. Injection of gonadotrophin releasing hormone 100 ug IV in this patient produced an LH rise from 4.9 to a maximum of 18.5 IU/l at 60 minutes and an FSH rise from 22.7 to >40 IU/l at 20 and 60 minutes. No patient in this series had Cushing's syndrome.

Table Clinical details and pre-operative plasma hormone levels in 24 patients with pituitary tumours

Patient No.	Age (yr)	Sex	Clinical presentation	Plasma hormone levels	
<i>Prolactinomas</i>					
				<i>PRL mU/L</i> (60–220 M:80–360 F)	
1	35	F	Chiasm. Comp.	18,250	
2	63	M	Chiasm. Comp.	517,000	
3	33	F	Amen./Infert.	15,600	
4	26	F	Chiasm. Comp.	2,600	
5	41	F	Amen./Infert.	200* (> 5,000)	
<i>GH secreting</i>					
				<i>PRL mU/L</i>	<i>GH mU/L</i>
6	68	F	Acromegaly	214	21.7
7	61	F	"	131	23.1
8	47	M	"	174	11.8
9	48	M	"	220	24.2
<i>Mixed (GH + PRL)</i>					
10	41	F	Acromegaly	2,240	> 32.0
11	49	M	"	36* (44,000)	8.8*
<i>"Functionless"</i>					
				<i>PRL mU/L</i>	<i>FSH IU/L</i> <i>LH IU/L</i>
					(3–12)
12	35	M	Chiasm. Comp.	1,100	3.2
13	60	M	"	965	10.8
14	75	M	"	149	2.2
15	35	M	"	670	—
16	70	M	"	803	4.8
17	42	F	"	1,650	2.7
18	18	M	"	220	4.6
19	45	M	"	640	1.3
20	49	M	"	820	0.9
21	60	M	"	970	7.2
22	63	M	"	615	8.9
23	44	M	"	960	1.8
24	50	M	Hypogonad.	197	22.7

Normal values are shown in brackets. GH levels represent the mean value during an oral glucose tolerance test. Starred values were obtained during treatment with bromocriptine, pretreatment values in brackets.

Methods

For maintenance tissue culture fragments of pituitary tumour were washed in normal saline and divided into explants 1–2 mm in diameter within 20 minutes of surgical removal. Two to eight explants of each tumour were placed in 4.0 ml Eagle's Minimum Essential Medium with Earle's salts and added penicillin 1000 U/ml, streptomycin 100 U/ml and glutamine 2 mM/l. After incubation for 24 hours at 37°C the supernatant was collected and stored at –20°C for hormone and NSE assay. The explants were weighed and secretion rates calculated in units/mg/24 hours.

GH and prolactin were assayed as previously described,¹⁴ LH and FSH were measured by radioimmunoassay using LH 68/40 and FSH IRP 78/549 as standards. The LH antiserum was F87/2 and showed the following cross-reactivity with other glycoproteins and their subunits calculated on a mass basis at 50% inhibition (LH = 100): FSH 0.8, TSH 2.3, HCG 9.7, β LH 15.8, β FSH 0.3, β TSH 0.4, β HCG 15.0, α HCG < 0.1. The FSH antiserum was M93/2 and cross-reactivity on the same basis (FSH = 100): TSH 0.2, β FSH 0.8, LH, HCG, β LH, β TSH, β HCG and α HCG < 0.1. LH and FSH for iodination were prepared by Professor W Butt.

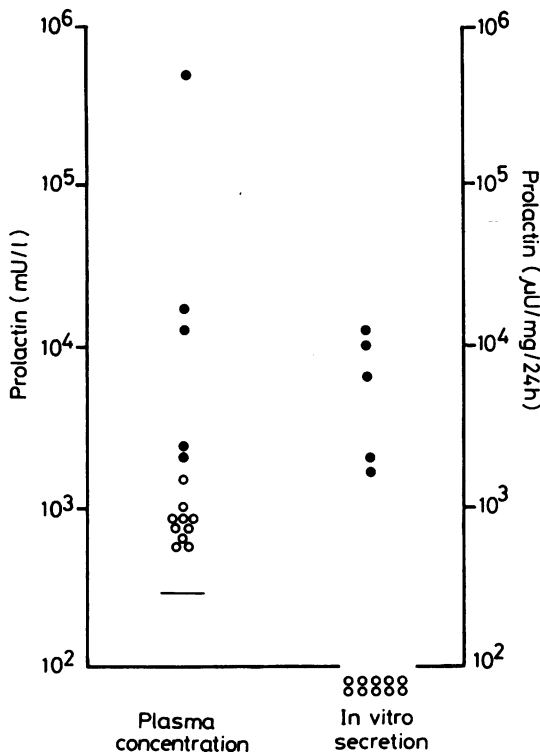


Fig 1 Plasma levels and IN VITRO secretion of prolactin in 15 patients with hyperprolactinaemia (log scales). High secretion rates (closed circles) correspond to patients with plasma prolactin of > 2000 mU/l. See text for details of secretion rates < 100 μ U/mg/24 h.

Between assay coefficient of variance for LH was 10.1% and for FSH 8.2%. Normal plasma values for prolactin, LH and FSH are shown in the table.

NSE was assayed by double-antibody radioimmunoassay using a kit kindly donated by Pharmacia Ltd. The NSE used as standard and tracer was purified from human brain by the method of Pählman *et al.*¹⁵ All samples in this study were run in the same assay. The detection limit was 6.5 μ g/l and intra-assay error was < 5%.

Results

Prolactin secretion

Fifteen patients were hyperprolactinaemic at the time of surgery but only five tumours showed high rates of prolactin secretion *in vitro*. Figure 1 shows the plasma prolactin levels and *in vitro* secretion rates in these 15 patients. In the five patients whose plasma prolactin ranged from 2,240–517,000 mU/l the *in vitro* secretion rate ranged from 1,785–13,447 μ U/mg/24 h. Four of these tumours were regarded as true prolactinomas and the fifth (Patient 10) as a mixed tumour. Pre-operative plasma prolactin was normal in two patients on bromocriptine (Patients 5 and 11) and *in vitro* prolactin secretion remained almost completely suppressed at 45 μ U/mg/24 h and 175 μ U/mg/24 h. Plasma prolactin levels of > 5,000 mU/l and 44,000 mU/l prior to bromocriptine suggest that these tumours were prolactin secreting and they are shown as a prolactinoma and a mixed tumour respectively in the table. In the 10 patients whose plasma prolactin ranged from 615–1650 mU/l *in vitro* prolactin secretion was undetectable in eight and negligible in two (33.9 and 28.9 μ U/mg/24 h). These tumours were regarded as “functionless”. Plasma prolactin was normal in three patients with “functionless” tumours and *in vitro* secretion rates were < 5 μ U/mg/24 h.

GH secretion

Tumours from patients with acromegaly secreted the expected large quantities of GH *in vitro* (fig. 2). In five untreated patients the secretion rate ranged from 1,665–28,344 μ U/mg/24 h. Patient 11 had persistently raised plasma GH levels on bromocriptine and an *in vitro* secretion rate of 2,324 μ U/mg/24 h.

Very low or undetectable GH secretion by five prolactinomas and 11 “functionless” tumours indicated that contamination of tumour tissue by normal pituitary was absent or minimal. Values ranged from undetectable to 3.1 μ U/mg/24 h in 13 cases and were 7.0, 19.2 and 28.0 μ U/mg/24 h in three.

LH and FSH secretion

In vitro secretion rates for LH and FSH are shown in fig 3. Secretion of both hormones by functionless tumours as a group was significantly increased compared with endocrine-active tumours ($p = < 0.01$,

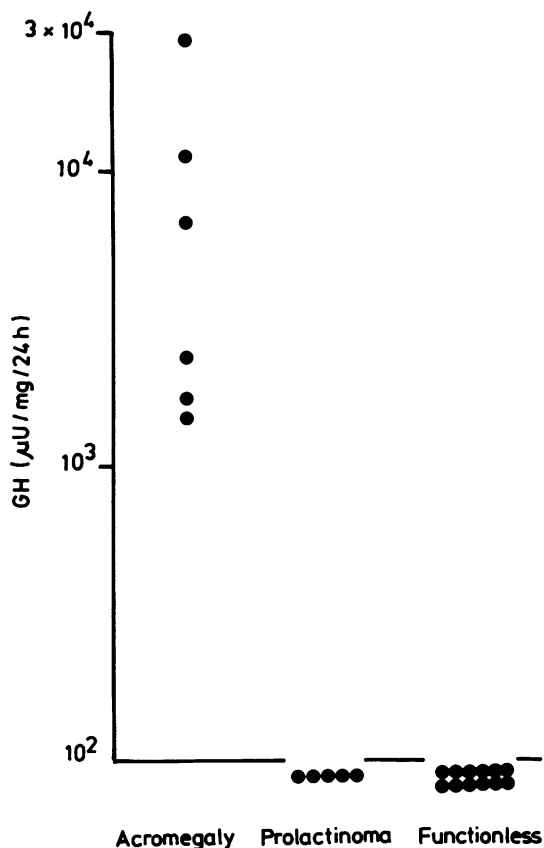


Fig 2 Growth hormone secretion IN VITRO by 22 tumours (log scale). See text for details of secretion rates < 100 μU/mg/24 h.

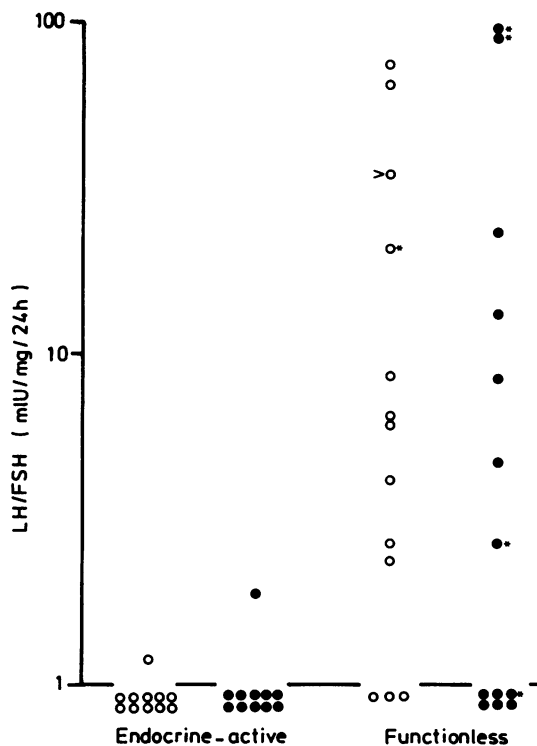


Fig 3 LH (open circles) and FSH (closed circles) secretion in vitro by 24 tumours (log scale). Starred circles correspond to high plasma LH and FSH levels. See text for details of secretion rates < 1.0 mIU/mg/24 h.

Mann-Whitney U test). There was no significant difference in the secretion rate of LH or FSH between prolactin and growth hormone secreting tumours. Low levels of LH secretion by endocrine-active tumours ranged from 0.1–1.1 mIU/mg/24 h. The secretion rate of LH by “functionless” tumours fell within this range in only three cases and was increased in 10. FSH secretion by five endocrine-active tumours was undetectable and ranged from 0.1–1.8 mIU/mg/24 h in six. The secretion rate by “functionless” tumours fell within this range in only six cases and was increased in seven. LH secretion was high in all 7 tumours showing high FSH secretion.

In spite of high gonadotrophin secretion *in vitro* plasma levels of LH were raised in only one patient and of FSH in only four, all of whom were male.

NSE secretion

Secretion rates for NSE are shown in fig 4. “Functionless” tumours as a group showed a significant increase in NSE secretion compared with endocrine-active tumours (p = < 0.01, Mann-Whitney U test). NSE secretion by two prolactinomas and three GH secreting tumours was undetectable and ranged from 0.5–2.8 ng/mg/24 h in the remaining six. Only four “functionless” tumours secreted NSE at these low levels, the values ranging from 4.4–> 200 ng/mg/24 h in the remaining nine. There was no correlation between the secretion rate of NSE and LH or FSH (p > 0.5, Wilcoxon’s t test).

Discussion

This study has shown that a majority of “functionless” tumours in an unselected series were associated with hyperprolactinaemia *in vivo* and increased secretion of gonadotrophins and NSE *in vitro* com-

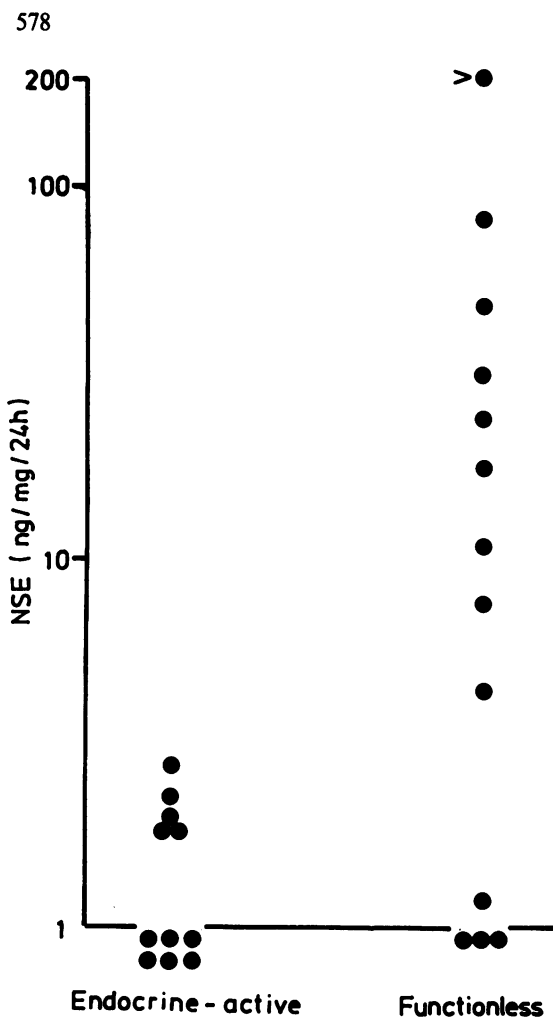


Fig 4 NSE secretion *in vitro* by 24 tumours (log scale). See text for details of secretion rates < ng/mg/24 h.

pared with prolactin and growth hormone secreting tumours.

The failure of 10 tumours from hyperprolactinaemic patients to secrete prolactin *in vitro* confirms the need to distinguish true prolactinomas from "functionless" tumours. Hyperprolactinaemia in patients with "functionless" tumours is probably due to a loss of the normal inhibitory control of prolactin secretion by hypothalamic dopamine acting as a physiological prolactin inhibiting factor (PIF). Several authors have reported hyperprolactinaemia due to craniopharyngiomas and other suprasellar lesions¹⁶⁻¹⁸ which cause increased prolactin secretion presumably by interfering with the delivery of PIF to the pituitary. Lundberg *et al*¹⁸ and Nabarro¹⁶

Lawton, Evans, Pickard, Perry, Davies

predicted that pituitary tumours other than prolactinomas could cause hyperprolactinaemia by the same mechanism. Recent reports of negative immunoperoxidase staining for prolactin in tumours from patients with modest hyperprolactinaemia have shown that "functionless" tumours may indeed masquerade as prolactinomas and can be regarded as "pseudoprolactinomas".¹⁹ The precise level of plasma prolactin required to confidently diagnose a true prolactinoma is not clear and it is probable that a degree of overlap exists between hyperprolactinaemia due to a prolactinoma and hyperprolactinaemia due to stalk compression.

Direct measurement of the prolactin secretion rate *in vitro* is likely to be more accurate in making this distinction than the qualitative assessment given by immunoperoxidase staining. In the present study two tumours from patients with prolactin levels of 2,240 mU/l and 2,600 mU/l were clearly true prolactinomas *in vitro*, whereas prolactin secretion was absent or negligible if plasma prolactin was <1,650 mU/l. It may be concluded that a patient with a pituitary macroadenoma and a plasma prolactin of <2000 mU/l is most unlikely to have a true prolactinoma. The distinction between prolactinomas and pseudoprolactinomas is clinically important because "functionless" tumours rarely regress on treatment with bromocriptine,²⁰ even though prolactin levels are suppressed. A combination of tissue culture and immunoperoxidase staining would provide the most accurate diagnosis of true prolactinomas and predict which tumours are likely to respond to bromocriptine following an incomplete surgical removal.

Our finding of increased FSH and/or LH by 10 of 13 "functionless" tumours supports the view of Mashiter *et al*¹³ that gonadotrophin secretion by these tumours is more common than previously supposed. Secretion of the α subunit of glycoprotein hormones by "functionless" tumours²¹ is well recognised but is not specific in that growth hormone and prolactin secreting tumours also secrete α subunit.²² We have shown that the secretion of whole immunoreactive LH and FSH on the other hand is significantly greater by "functionless" tumours than by endocrine-active tumours. It seems unlikely therefore that LH or FSH in the tissue culture supernatant is derived from islands of normal gonadotrophs which have become engulfed by tumour tissue. Nor is it likely that the immunoreactive LH and FSH assayed in this study can be explained by cross-reactivity of the antisera with α subunit since cross-reaction with α HCG is <0.1 for both antisera.

There are, however, reasons to doubt that LH and FSH are the major hormones secreted by "functionless" tumours. Firstly, in agreement with Mashiter *et al*,¹³ we noted that the secretion rates of LH and

FSH were much lower than the rates for prolactin and growth hormone secretion by endocrine-active tumours. In addition, Trouillas *et al*¹¹ reported that the amounts of LH and FSH which could be extracted from tumours in their series were lower than the amounts of LH and FSH extracted from normal pituitary. Secondly, immunoperoxidase staining has shown that only a minority of tumour cells contain FSH or LH.¹³ Thirdly, the expected increase in plasma LH or FSH was uncommon in our patients and often marginal when present. This was particularly true of LH, 10 tumours secreting an excess *in vitro* but only one patient showing a raised plasma LH. In the case of FSH there was no clear correlation between high secretion rates *in vitro* and raised plasma levels (fig 3). Preoperative plasma levels of LH and FSH were available for only one patient in the study by Mashiter *et al*.¹³ Although secretion of immunoreactive LH and FSH by "functionless" tumours undoubtedly occurs we are therefore reluctant to regard these tumours as "gonadotrophinomas." Indeed only one patient in this series (Patient 24) may have harboured a true FSH secreting tumour in view of the recognised association with hypogonadism²³ and the clear cut increase in plasma FSH with an exaggerated FSH response to the releasing hormone.

The significance of NSE in neuroendocrine cells is unknown but the high level of NSE secretion by nine "functionless" tumours provides biochemical evidence for their metabolic activity. Although NSE is uniformly present in endocrine-active tumours by immunocytochemical staining,⁸ low or undetectable levels of NSE secretion by the endocrine-active tumours in this study suggest that prolactin and GH secreting cells are not a major source of NSE. The lack of correlation between NSE and LH or FSH secretion does not support the view that gonadotrophs are the major source of NSE in "functionless" tumours. We have also measured NSE secretion by two tumours from patients with Cushing's syndrome. The results were not included in this series because ACTH levels were not measured, but NSE secretion was low in both cases. NSE secretion by "functionless" tumours was not universally increased since the values in four tumours were comparable to those of endocrine-active tumours. Present evidence suggests however that a high rate of NSE secretion is characteristic of the cell type from which most "functionless" tumours derive.

Our preliminary studies of plasma NSE levels show occasional high values in patients with pituitary tumours but it is unlikely that plasma NSE can be used to monitor therapy in "functionless" tumours. Nevertheless, measurement of NSE secretion *in vitro* should provide a useful biochemical marker in further studies of these tumours whose putative hormonal

activity remains an enigma.

We are grateful to Dr PJ Wood for LH and FSH assays.

References

- ¹ Kovaks K, Horvath E, Ryan N, Ezrin C. Null cell adenoma of the human pituitary. *Virchow's Arch Pathol Anat* 1980;**387**:165-74.
- ² Schmechel D, Marangos PJ, Brightman M, Goodwin FK. Brain enolases as specific markers of neuronal and glial cells. *Science* 1978;**199**:313-5.
- ³ Facer P, Polak JM, Marangos PJ, Pearse AGE. Immunological localization of neurone-specific enolase (NSE) in the gastrointestinal tract. *Proc R Microscop Soc* 1980;**15**:113-4.
- ⁴ Schmechel D, Marangos PJ, Brightman M. Neurone-specific enolase is a molecular marker for peripheral and central neuroendocrine cells. *Nature* 1978;**276**:834-6.
- ⁵ Cole GA, Polak JM, Wharton J, Marangos P, Pearse AGE. Neurone-specific enolase as a useful histochemical marker for the neuroendocrine system of the lung. *J Pathol* 1980;**132**:351-2.
- ⁶ Tapia FJ, Polak JM, Barbosa AJA, *et al*. Neurone-specific enolase is produced by neuroendocrine tumours. *Lancet* 1981;**1**:808-11.
- ⁷ Marangos PJ, Schmechel D, Parma AM, Clarke RL, Goodwin FK. Measurement of neurone-specific (NSE) and non-neuronal (NNE) isoenzymes of enolase in rat, monkey and human nervous tissue. *J Neurochim* 1979;**33**:319-29.
- ⁸ Van Noorden S, Polak JM, Robinson M, Pearse AGE, Marangos PJ. Neuron-specific enolase in the pituitary gland. *Neuroendocrinology* 1984;**38**:309-16.
- ⁹ Royds JA, Timperley WR, Taylor CB. Levels of enolase and other enzymes in the cerebrospinal fluid as indices of pathological change. *J Neurol Neurosurg Psychiatry* 1981;**44**:1129-35.
- ¹⁰ Royds JA, Davies-Jones GAB, Lewtas NA, Timperley WR, Taylor CB. Enolase isoenzymes in the cerebrospinal fluid of patients with diseases of the nervous system. *J Neurol Neurosurg Psychiatry* 1983;**46**:1031-6.
- ¹¹ Trouillas J, Girod C, Sassolas G, *et al*. Human pituitary gonadotrophic adenoma; histological, immunocytochemical and ultrastructural and hormonal studies in eight cases. *J Pathol* 1981;**135**:315-36.
- ¹² Kovaks K. Light and electron microscopic pathology of pituitary tumours: immunohistochemistry. In: Black PM, Zervas NT, Ridgeway EC, Martin JB, eds. *Secretory Tumours of the Pituitary Gland. Progress in endocrine research and therapy, vol. 1*. New York. Raven Press 1984:365-75.
- ¹³ Mashiter K, Adams E, Van Noorden S. Secretion of LH, FSH and PRL shown by cell culture and immunocytochemistry of human functionless pituitary adenomas. *Clin Endocrinol* 1981;**15**:103-12.

- ¹⁴ Lawton NF, Evans AJ, Weller RO. Dopaminergic inhibition of growth hormone and prolactin release during continuous in vitro perfusion of normal and adenomatous human pituitary. *J Neurol Sci* 1981; **49**:229–39.
- ¹⁵ Pählman S, Esscher T, Bergvall P, Odelstad L. Purification and characterization of human neurone-specific enolase: development of a radioimmunoassay. *Tumour Biol*, In press.
- ¹⁶ Nabarro JDN. Pituitary prolactinomas. *Clin Endocrinol* 1982; **17**:129–55.
- ¹⁷ Kappala LP, Molitch ME, Post KD, *et al.* Galactorrhoea, oligo/amenorrhoea and hyperprolactinaemia in patients with craniopharyngiomas. *J Clin Endocrinol Metab* 1980; **51**:798–800.
- ¹⁸ Lundberg PO, Osterman PO, Wide L. Serum prolactin in patients with hypothalamic and pituitary disorders. *J Neurosurg* 1981; **55**:194–9.
- ¹⁹ Grossman A, Besser GM. Prolactinomas. *Br Med J* 1985; **1**:182–4.
- ²⁰ Barrow DL, Tindall GT, Kovaks K, Thorner MO, Horvath E, Hoffman JC. Clinical and pathological effects of bromocriptine on prolactin-secreting and other pituitary tumours. *J Neurosurg* 1984; **60**:1–7.
- ²¹ Kourides IA, Weintraub BD, Rosen SW, Ridgeway EC, Kliman B, Maloof F. Secretion of alpha subunit of glycoprotein hormones by pituitary adenomas. *J Clin Endocrinol Metab* 1976; **43**:97–106.
- ²² MacFarlane IA, Beardwell CG, Shalet SM, Darbyshire PJ, Hayward E, Sutton ML. Glycoprotein hormone alpha subunit secretion by pituitary adenomas: Influence of external irradiation. *Clin Endocrinol* 1980; **13**:215–22.
- ²³ Woolf PD, Schenk EA. An FSH producing tumour in a patient with hypogonadism. *J Clin Endocrinol Metab* 1974; **38**:561–8.