TISSUE CULTURE STUDIES ON HUMAN PITUITARY TUMOURS: RADIOIMMUNOASSAYABLE ANTERIOR PITUITARY HORMONES IN THE CULTURE MEDIUM

By
Loren G. Lipson, Inese Z. Beitins, Paul D. Kornblith, Janet W. McArthur, Henry G. Friesen, Bernard Kliman and Raymond N. Kjellberg

ABSTRACT

A tissue culture study was undertaken to determine if human non-functioning pituitary tumours secrete polypeptide anterior pituitary hormones in vitro and to study the spectrum of hormone release by functioning pituitary neoplasms. Fragments from 48 human pituitary tumours (from patients – 2 with Cushing’s disease, 1 with Nelson’s syndrome, 5 with amenorrhoea-galactorrhoea, 10 with acromegaly and 30 with non-functioning pituitary tumours) and three normal human anterior pituitary glands (controls) were placed in tissue culture immediately after surgery. The in vitro release of human growth hormone (HGH), prolactin (Prl), thyrotrophin (TSH), adrenocorticotrophin (ACTH), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by radio-immunoassays at the end of one week in culture. Clinical and pathological data were compared to hormone release patterns.

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In the culture media from control pituitaries the concentrations of the six hormones tested were 100 to 10,000 times greater than in peripheral blood. The medium surrounding the fragments from functioning pituitary tumours contained the following: a) Acromegaly – high levels of HGH and variable concentrations of the other hormones. b) Cushing’s disease – ACTH and Prl predominantly. c) Amenorrhoca-galactorrhoca syndrome – prolactin in 4 out of 5 patients, all six polypeptides in one patient. In the media from the 30 patients diagnosed as having non-functioning pituitary tumours, 60% of the samples contained at least one hormone at a concentration similar to that of the controls and 100% of the samples contained detectable quantities of at least one hormone.

Much of the knowledge of human pituitary function has, of necessity, been gained indirectly by comparing the plasma and urine levels of radioimmunoassayable hormones during basal, stimulated and suppressed states. In animals, direct approaches to the pituitary gland have included organ perifusion, venous catheterization, and organ and tissue culture (Tashjian et al. 1968; Steinberger et al. 1973). In the human, direct approaches have been limited to investigations of pituitary hormone content of glands obtained from abortuses or cadavers, from carvenrous sinus catheterization during pituitary surgery and from tissue culture studies on foetal pituitaries or on a few adult human pituitaries removed at surgery (Nicoll 1972; Pasteels 1972; Zimmerman et al. 1974; Gala 1971; Pierson et al. 1973; Gitlin & Biasucci 1969; Kohler et al. 1969; Batzdorf et al. 1971; Teraoka 1972; Peillon et al. 1973; Espinoza & Neuss 1974; Skyler et al. 1977).

Foetal pituitary cells have been shown to synthesize and release polypeptide hormones in vitro as well as to respond to the presence of exogenous hormones and releasing factors (Siler-Khodr et al. 1974). The few studies involving tissue from normal and adenomatous adult human pituitaries have shown the release of as many as three hormones from a single specimen and de novo synthesis of one hormone (Kohler et al. 1971).

We have had the opportunity to investigate the release of anterior pituitary hormones into culture medium by 3 adult human pituitary glands and 48 pituitary adenomas removed at surgery. The objectives of this study were to determine the hormone release patterns in vitro of non-functioning pituitary tumours (so-called chromophobe adenomas) and to study polypeptide hormone production by functioning pituitary neoplasms.

METHODS

Patient selection for surgery

Twenty-three women and 28 men, ages 19 to 74, requiring hypophysectomy were included in this study. Criteria for surgery included one or more of the following:
decreased visual fields; enlarged or irregularly shaped sella turcica with suprasellar extension of the tumour mass demonstrated by pneumoencephalography, and excessive hormone secretion. It should be emphasized that this study was retrospective. In most instances only those clinical studies were performed that were needed to establish the diagnosis, no uniform protocol being utilized.

Based on the clinical appearance, radiological findings and hormone determinations, the patients were classified as follows: 2 Cushing’s disease, 1 Nelson’s syndrome, 5 amenorrhoea-galactorrhoea syndrome, 10 acromegaly and 30 non-functioning pituitary tumours. Three patients with breast or prostate carcinoma, who had previously undergone gonadectomy, were subjected to palliative total hypophysectomy.

Surgery

The sella or tumour mass was approached by either the transphenoidal or the transfrontal route. Microsurgical dissection was performed on the sella contents and tissue fragments were removed under sterile conditions. The tissue was divided in the operating room so as to yield homogenous portions for pathological evaluation and tissue culture.

Pathology

The pituitary fragments were fixed in 10% formalin, dehydrated, sectioned and stained with haematoxylin and eosin. In selected cases, PAS-orange G staining was also used. The criteria for the pathological diagnoses included the absence of neural tissue and presence of eosinophilic or basophilic staining of the pituitary cells. If the cells failed to accumulate stain, they were classified as chromophobe.

Tissue culture studies

After weighing, the pituitary fragments were gently agitated in three changes of Ham’s F 10 nutrient medium (Ham & Puck 1962) containing 15% foetal calf serum. The tissue was finely diced into cubes of 1 mm³. Ten of these were placed in a 25 cm² tissue culture flask and 1 ml of medium was added per 10 mg wet weight of pituitary tissue. Each flask was then placed in a water jacketed tissue culture incubator at 37°C. Cellular growth was observed with a phase contrast light microscope. After 7 days in culture, the medium was removed from each flask, centrifuged at 2000 × g for 5 min to remove particulate matter, divided into aliquots and stored frozen at −20°C for subsequent hormonal assay.

Hormonal measurements

**HGH and Prl.** – Double antibody techniques described by Beck et al. (1965) for HGH and a modification of the method reported by Hwang et al. (1971) for Prl were used. Immunochemical grade HGH (NIH 2.60 U/mg from NIAMDD) and a human Prl preparation (Friesen-57) were used for iodination and standards. The HGH was iodinated by the chloramphenicol T procedure (Hunter & Greenwood 1962) and Prl by the lactoperoxidase procedure (Thorell & Johansson 1972). Inter-assay variability was less than 15% and intra-assay variation less than 7% for both assays. The sensitivity was 1 ng/ml for the HGH assay and 3 ng/ml for the Prl assay.

**ACTH.** – ACTH was measured by radioimmunoassay following extraction and absorption onto glass micro-beads by the method of Ratcliff & Edwards (1971). Reagents were supplied by Amersham-Searle. The detection limit was 0.5 ng/ml and intra-assay
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<th>TSH (μU/ml)</th>
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<sup>a</sup> Mixed eosinophilic and chromophobe adenoma.

variability at a concentration of 2.5 ng/ml was 11.2 %. Inter-assay variability was 21 %.

TSH. TSH was measured by a double antibody method (Hershman & Pittman 1971) using reagents supplied by Beckman, Inc. The method was capable of detecting 1.5 μU/ml TSH for assay aliquot of 200 μl. The coefficient of variation for an assay
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**Non-functioning pituitary tumours**

Standard with a concentration of 15.5 µU/ml was 6.2%. Cross-reactivity for human LH was 1 to 2% and for human FSH was 2 to 5%. Inter-assay variability for 5 successive assays was 11%.

**LH and FSH.** – Both LH and FSH were measured by the double antibody radioimmunoassay technique developed by Midgley (1966, 1967). Highly purified LH
10 000 IU/mg protein and FSH 5000 IU/mg protein compared to the 2nd International Reference Preparation of Human Menopausal Gonadotropin were obtained from A. B. Kabi, Stockholm (Roos 1968) and were used for iodination and as standards. The limit of sensitivity for the LH system was 125 pg and for FSH was 62.5 pg per sample, respectively. The intra-assay variability for LH was 4.5% while the inter-assay coefficient of variation was 11.4%. The intra-assay variability for FSH was 3.3% and the inter-assay coefficient of variation was 13.9%.

None of the six hormones were detectable in control culture medium.

RESULTS AND DISCUSSION

Light microscopy studies of the excised specimens

Permanent sections of all pituitary specimens were reviewed by one examiner and the pathological diagnoses determined (Table 1). The pituitary cells from the 3 agonadal patients who had palliative surgery for carcinoma revealed no pathological abnormalities and were classified as normal.

The specimens from patients clinically diagnosed as having Nelson’s syndrome, Cushing’s disease and amenorrhea-galactorrhoea syndrome stained poorly with eosin and haematoxylin. Of the 10 patients clinically diagnosed as having acromegaly, the pathological diagnoses were 4 eosinophilic adenomas, 2 mixed eosinophilic and chromophobe adenomas and 4 chromophobe adenomas. All 30 specimens from patients diagnosed as having non-functioning pituitary tumours stained poorly and were called chromophobe adenomas.

The staining characteristics of pituitary cells are dependent upon the number, size and type of secretory granules within the cytoplasm. Cells having few or no granules do not retain haematoxylin and eosin and are classified as “chromophobe”. Tumour cells which secrete hormone, however, may retain few storage granules because of active secretion (cases 4–11), making these cells indistinguishable from those which are non-functioning using light microscopy. In the cases of patients with acromegaly (cases 12–21), the heterogeneity of pathological diagnoses may reflect cells having different HGH secretory rates or be simply a tissue sampling bias with tumour and normal cells present.

Light microscopy studies of pituitary cells grown in culture for one week

Although cellular growth was slow during the first week in culture, several morphological changes were noted. First, there was an outgrowth of bipolar cells from the periphery of the explants leading to the appearance of a palisade effect. At the end of the week, round epithelioid cells with large nuclei and nucleoli were noted adjacent to the explanted tissue. The bipolar cells are thought to represent fibroblasts and the epithelioid cells, pituitary epithelial cells. In addition, there were small clusters or rosettes of epithelioid cells which could be easily dislodged from the surface of the culture flask. Similar
findings have been noted in the culture media of human pituitary fragments by other investigators (Batzdorf et al. 1971; Teraoka 1972; Tixier-Vidal 1975) and in cultures of other endocrine tissues such as the parathyroid and the thyroid glands (Lissitzky et al. 1971).

Radioimmunoassayable hormone concentrations in the culture media from human pituitary cells

After the pituitary cells had been in culture for one week, the medium was removed and concentrations of HGH, Prl, ACTH, TSH, LH and FSH were measured by specific radioimmunoassays (Table 1). It is recognized that the hormone concentrations in the medium represent synthesis and release from pituitary cells, release of preformed hormone, hormone leakage from cells as well as hormonal degradation within the medium.

Carcinoma of the breast or prostate. – Two men (ages 64 and 68 years old) with carcinoma of the prostate and one woman (46 years old) with carcinoma of the breast underwent transsphenoidal hypophysectomy for palliation after previous gonadectomy had failed to alter the course of their disease. All 6 anterior pituitary hormones were released into the culture medium in substantial concentrations. The concentration of Prl was highest in the woman (2800 ng/ml) but was easily measurable in the men (160 and 500 ng/ml). HGH was released in quantities (20 000–80 000 ng/ml) about 10 000 times greater than normal peripheral plasma concentrations. ACTH concentrations ranged between 5 and 75 ng/ml. TSH levels were between 50 and 600 µU/ml. LH was in the range > 200–2000 ng/ml and FSH between 50 and 200 ng/ml. The release of all 6 hormones implied that these polypeptides were either previously stored in the tissue fragments or newly synthesized and released into the tissue culture medium. Media hormone concentrations from these 3 gonadal patients served as our control values. Similar findings have been reported in normal pituitaries by Batzdorf et al. (1971) for HGH, Teraoka (1972) for HGH and LH, Kohler et al. (1969) for HGH, LH and TSH, Gala (1971) and Pasteels (1972) for Prl. In addition, in the case of HGH, de novo synthesis has been shown to occur in the culture of normal pituitary cells (Kohler et al. 1971).

The concept that pituitary cells are capable of storing large quantities of hormones gains support from the following studies: Conway et al. (1969) demonstrated HGH, LH and TSH concentrations in intrapituitary sinusoid blood at the time of palliative hypophysectomy in 18 patients were 20 to 90 times higher than those in the peripheral circulation. In acute 1 h incubation experiments with individual normal human pituitary cells, Espinoza & Neuss (1974) found that the 6 anterior pituitary hormones assayed were generously released. The most extensive studies of human anterior pituitary cells in culture have utilized foetal glands (Gitlin & Biasucci 1969; Siler-Khodr et al. 1974). Siler-
Khodr et al. (1974) obtained anterior pituitary glands from 40 foetuses, 4 to 40 weeks of gestational age. With time in culture, the concentrations of HGH, ACTH, TSH, LH and FSH decreased but those of Prl and MSH increased. These findings were interpreted as a response of the pituitary cells to the lack of hypothalamic releasing and inhibitory hormones, respectively. Further, de novo synthesis of Prl, TSH, LH and FSH was demonstrated by radiolabelled amino acid incorporation. The presence of large quantities of radioimmuno-assayable hormones in the culture medium of control human adult pituitary cells indicate that they may behave in vitro like those of the foetuses.

Cushing’s disease and Nelson’s syndrome. – The 2 patients with Cushing’s disease were men, 28 and 33 years of age. One patient (case 4) had an invasive, recurrent pituitary tumour. The culture medium from his pituitary cells contained 1320 ng/ml of ACTH which exceeded the concentrations in the media from control pituitary cells (5–75 ng/ml). The second patient (case 5) had early Cushing’s disease and the ACTH concentration in the medium surrounding his pituitary cells was 11 ng/ml. In both cases the Prl concentrations were markedly elevated (16 250 and 56 000 ng/ml). HGH, TSH, LH and FSH were present in low concentrations (Table 1). Neither patient had gynaecomastia or galactorrhoea. There are two reports in the literature of patients with concurrent Cushing’s disease, pituitary tumour and galactorrhoea (Levin et al. 1959; Young et al. 1967). Studies of prolactin physiology in Cushing’s disease, however, do not explain these findings (Krieger et al. 1976; Kleinberg et al. 1977).

The tumour from the patient with Nelson’s syndrome and galactorrhoea (case 6) released elevated levels of ACTH and Prl, and also released the other four hormones at levels comparable to or above those of control cultures. In the only other reported study, Teraoka (1972) measured ACTH in culture medium of a tumour from a patient with Nelson’s syndrome but could detect only 1 ng/ml.

Amenorrhoea-galactorrhoea syndrome. – Five women ages 20 to 32 years were clinically diagnosed as having the amenorrhoea-galactorrhoea syndrome. When tested pre-operatively, these women had hyperprolactinaemia, impaired gonadotrophin secretion and one (case 11) had hypothyroidism. They had normal a.m. plasma cortisol levels (15 to 18 μg/100 ml) and urinary 24 h 17-hydroxycorticoid excretion (3.3 to 7.5 mg). The culture medium surrounding their pituitary tumour cells contained only high concentrations of Prl (18 000 to 122 000 ng/ml) in 4 cases. In one patient (case 10), the tumour cells produced high concentrations of all 6 hormones.

Acromegaly. – Tissue culture media surrounding tumour fragments from 5 women and 5 men (ages 22 to 53) with acromegaly contained concentrations of HGH ranging from 3200 to 74 000 ng/ml. These concentrations were not
higher than those released by cultures from our controls (from agonalad pa-
tients) and, in fact, were lower in some cases. This finding is consistent with
a high turn-over rate and less storage of HGH in the tumours while in controls
HGH is accumulated and release processes are under more inhibitory control
than in the tumours. In addition, Prl was present in measurable concentrations
(7 to 9000 ng/ml) in all culture media. No correlation between the concentra-
tions of Prl and HGH was noted. This is consistent with the hypothesis that
Prl and HGH originate from two distinct cell types. ACTH and TSH concen-
trations varied widely (undetectable to 800 ng/ml for ACTH and undetect-
able to 950 μU/ml for TSH). Gonadotrophins were low (compared to control
cultures from agonalad patients) in all cases except for case 12. This patient
had progressive acromegaly with the pathological diagnosis of an eosinophilic
adenoma, yet the pituitary cells in culture released high concentrations of all
the hormones measured.

The tumours from patients with acromegaly have been extensively studied
(Kohler et al. 1969, 1971; Batzdorf et al. 1971; Teraoka 1972; Peillon et al.
1973; Espinoza & Neuss 1974; Skyler et al. 1977). In most instances, however,
following in vitro culturing only HGH and gonadotrophins have been mea-
ured by radioimmunoassay. Generally the gonadotrophin concentrations have
been low with a great variability in the HGH concentrations.

Non-functioning pituitary tumours. – Thirty patients had non-functioning
pituitary tumours (11 women and 19 men, aged 19 to 74 years). After one week
in culture, easily detectable quantities of Prl were present in cases 22 to 26.
The Prl concentration within the culture media was substantial (3750 to
50 000 ng/ml). These tumours were removed from 4 men (ages 19, 30, 36 and
61) and one woman (age 40) who did not have galactorrhoea. Three of the
men (cases 24, 25 and 26) had hypogonadism at the time of surgery, two (cases
25 and 26) had gynaecomastia and one (case 24) had hypothyroidism.

The remaining 25 media from patients with non-functioning pituitary tu-
mours (cases 27 to 51) were of interest because all contained detectable con-
centrations of at least one hormone (12/o had 1 hormone detectable; 36/o –
to 2 hormones; 25/o – 3; 12/o – 4; 8/o had 5; and 8/o – 6 hormones) and 52/o
of these contained high concentrations of at least one hormone in the range
of the control cultures (48/o had none; 16/o had one hormone at control level;
28/o had 2; 4/o – 3; 0/o – 4; 4/o – 5 hormones; 0/o had 6). Of these 25
specimens assayed for the six hormones, 48/o of the media had detectable
quantities of Prl; 32/o had HGH; 36/o had ACTH; 16/o had TSH; 96/o
had LH and 72/o had FSH. Thus gonadotrophins were the most frequently
found hormones. Although the pituitary cells failed to retain dye, they proved
capable of hormone release. Several possibilities exist. The tumour specimen
in culture could have contained some normal pituitary cells which continued
to function, or the chromophobe-appearing cells themselves may be secreting hormone with a rapid turnover rate.

Other investigators have attempted to assay hormones in the culture media from chromophobe adenomas with variable results (Kohler et al. 1969; Batzdorf et al. 1971; Teraoka 1972). Low concentrations of HGH and TSH have been demonstrated. Gonadotrophin concentrations were low in the media from 17 adenomas but high in two such cases (from a 35 year old woman and a 25 year old man) (Kohler et al. 1969; Teraoka 1972). Chromophobe adenomas represent a wide spectrum of tissue types with varying degrees of hormonal activity, all having in common an absence of staining by haematoxylin and eosin at the time of surgery. This diversity precludes comparative studies.

Finally, it should be noted that one patient with Nelson's syndrome (case 6), one with amenorrhoea-galactorrhoea syndrome (case 10) and one with acromegaly (case 12) had pituitary tumours which in culture released high concentrations of all six hormones assayed. Yet each patient presented clinically with a specific syndrome without indication of excessive biologic activity of the other peptides released in excess. One possibility is that some of the radioimmunoassayable hormones may lack biologic activity. Alternatively, the tumours may have consisted predominantly of one cell type, but due to sampling bias normal cells were included in the culture and released all six hormones. Finally, it is possible that a more primitive cell type capable of producing several anterior pituitary hormones may have been present in the explants. In such circumstances exaggerated concentrations within the culture medium may be attributed to release of stored hormone which is not released in vivo.

These studies confirm the facts that human pituitary tumour cells can be grown in tissue culture and that multiple hormones in the medium can be determined by radioimmunoassay. Culture media from control pituitary cells contained HGH, Prl, ACTH, TSH, LH and FSH in easily measurable quantities. The culture media from tumours of patients with amenorrhoea-galactorrhoea and acromegaly had high concentrations of Prl and HGH, respectively. However, all of the specimens derived from patients with non-functioning pituitary adenomas likewise released detectable amount of radioimmunoassayable hormones into the culture medium. Thus measurements of hormone concentrations within the culture medium of pituitary cells may provide more information than the staining characteristics conventionally employed to classify these tumours.

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