Growth Hormone and Prolactin Secretion of Pituitary Adenoma

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Summary

Tissue culture of 20 pituitary adenomas (four acromegalias, 15 non-functioning tumors, one Forbes-Albright syndrome) was performed with the Maxmow double cover glass assembly. Growth hormone and prolactin in the medium were measured during the culture. Morphological feature in vitro was also compared with that of the original tumor.

In chromophobe adenomas of clinically non-functioning, tumor cells had round secretory granules of not only 100, but also 300 μμ in diameter. The study of their tissue culture revealed that these tumor cells were secreting growth hormone in all the cases.

In acromegalic patients, the culture medium a month later still contained more than 1000 ng/ml of growth hormone and also many secretory granules remained in the cultured cells. Thus, it was suspected that the tumor cells in acromegalic patients had autonomous secretory functions of growth hormone.

In pituitary adenomas of Forbes-Albright syndrome and even non-functioning or acromegaly, prolactin secretion from tumor cells was demonstrated. Ovoid, ellipsoid, or irregular secretory granules of 500 to 1000 μμ in acromegalic patients and 150 to 500 μμ in the other cases were observed with an electron microscope.

Key word: pituitary adenoma, acromegaly, Forbes-Albright syndrome, growth hormone, prolactin, tissue culture.

Introduction

Pituitary adenomas are usually classified into three categories: basophilic, eosinophilic, and chromophobe adenomas by their histological properties with haematoxylin and eosin stain. From the endocrinological standpoint of view, they are divided into two groups, hormonally functioning and nonfunctioning tumors.

The concepts that eosinophilic adenomas are associated with acromegaly or gigantism, basophilic with Cushing’s disease, and chromophobe with hypopituitarism has been recognized for quite some time.

In recent years, however, chromophobe adenomas which had been confirmed histologically, were shown to be associated with acromegaly, Cushing’s syndrome30), hyperthyroidism15), Forbes-Albright syndrome9), and FSH producing tumor37). Thus, it appears reasonable to assume that some chromophobe adenomas are hormonally functioning.

Many electron microscopic studies of normal pituitary glands3,7,10,19,23,27,29) and pituitary adenomas13,14,18,31,32,36) have been reported. Luse24) and other investigators13,32) described the sizes of secretory granules to be mainly 300 μμ in both eosinophilic and basophilic adenomas and 100 μμ in most chromophobe adenomas. They also reported some chromophobe adenomas with secretory granules of 300 μμ or both 100 and 300 μμ.

On the other hand, histochemical identification and hormone assay in the medium of tissue culture have been performed to investigate the hormone secretion of pituitary adenomas. Secretion or synthesis of hormones was demonstrated only in a few cases of chromophobe adenomas4,21).

To elucidate the discrepancy between these morphological and hormone assay studies on pituitary adenomas, human growth hormone (hGH) and human prolactin (hPr) were measured in the medium of tissue culture with the Maxmow double cover glass assembly.

The autonomy of hGH secretion in acromegaly is also discussed in this report.

Materials and Method

Tumor tissues from four acromegalias, 15
non-functioning tumors and one Forbes-Albright syndrome were removed by surgery and cultured by the Maximow method. Brain tumors other than pituitary adenoma and mouse cerebellum were also cultured as controls.

Tumor tissues were washed twice with Hank’s BSS and cut into 0.5 mm³ pieces. Aliquots of two fragments were explanted on to a coverslip previously coated with a gel of reconstituted rat-tail collagen and fed a single drop of nutrient medium (0.04-0.045 ml), consisting of 50% Eagle’s minimum essential medium, 25% horse serum and 25% Hank’s BSS, also containing 600 mg% glucose, were sealed with paraffin. All preparations were incubated at 37°C. The culture medium was renewed two or three times a week. The exchanged medium was kept frozen until hormones were assayed. As a rule, 32 slides were made for one case. Histological examinations with May-Gimsa or with Haematoxylin-Eosin stains were carried out at various stages of the culture after daily dynamic observations under the phase contrast microscope. For electron microscopic observation, samples were fixed with 2.5% glutaraldehyde and 1% osmic acid, embedded in vestopal or epon and stained with lead citrate and uranyl acetate. Growth hormone and prolactin levels in the culture medium were determined by radioimmunoassay using the double antibody method. The standards employed for the assay of hGH was Wilhelmi (NIH HS-1895) and prolactin was NIH Friesen #1. Standard curve using medium was in complete accordance with assay using a buffer. In this system, GH sensitivity was 1 ng/ml and prolactin was 2.5 ng/ml.

### Results

1. Morphological observations

   1) in vivo
      a) light microscopic observation
      Histological examination of 20 pituitary adenomas with haematoxylin and eosin stain disclosed that two of four acromegalic patients were eosinophilic and the others were mixed adenomas. One case of Forbes-Albright syndrome and 15 cases of non-functioning tumors were chromophobe adenoma (Table 1).

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   b) electron microscopic observation
   Electron microscopic study of eight chromophobe adenomas revealed round secretory granules, 100 μm in diameter in all, and 300 μm in diameter in some cases. Two eosinophilic adenomas contained secretory granules of 300 to 500 μm in diameter diffusely in the cytoplasm. One mixed adenoma, case A3, showed two kinds of cells; one of them contained round secretory granules of about 300 μm like eosinophilic adenoma and the other ovoid, ellipsoid, or irregular secretory granules of 500 to 1000 μm (Fig. 1), which were similar to prolactin granules of human or animal lactotrophs.
   Case Cl of non-functioning and case FAI of Forbes-Albright syndrome had similar but smaller granules of 150 to 500 μm (Fig. 2). Electron micrograph revealed that these irregular granules were formed by the fusion of round granules (Fig. 3).

   2) in vitro
      a) light microscopic observation
Fig. 1. Electron micrograph, adenoma cells of acromegaly. STH: Growth hormone secreting cell with round secretory granules of about 300 μm in diameter. LTH: prolactin secreting cell with ovoid or irregular (right upper) secretory granules of 500 to 1000 μm. × 6500.

Fig. 2. Electron micrograph, adenoma cell of clinically non-functioning tumor. Ovoid or rod-shaped secretory granules of 150 to 250 μm in the cytoplasm. × 25000.
Under the phase contrast microscope, tumor cells partly showed a cord-like or acinous structure after one or two day of culture (Fig. 4). After three to four days, they formed tubular structures. At the stage of two or three weeks, culture cells showed similar histological structure to that of the original tumor (Fig. 5). After this stage, proliferation of the tubular structure was not vivid but further culture was stable for more than one month.

b) electron microscopic observation

Electron microscopic study of chromophobe adenomas revealed some difference between initial and late stages of the culture. As shown in Fig. 6, adenoma cells of three-day culture were rich in secretory granules of 100 and 300 m\( \mu \) in diameter, but after three weeks they disappeared from the cytoplasm (Fig. 7).

Ultrastructural changes during the culture in eosinophilic adenomas were different from those in chromophobe adenomas. That is, round secretory granules were rich even after three weeks. And they were larger than those of the original tumor. Furthermore, these granular cells had many microvilli and formed the follicular structure (Fig. 8).
II. Hormone levels in the culture medium

1) growth hormone
   a) chromophobe adenoma
      Radioimmunoassay of hGH in the culture medium revealed that the concentration was highly than 25 ng/ml in five cases while it was one to five ng/ml in the other five at the early stage of the culture of 10 chromophobe adenomas. However, hGH concentrations of control groups such as other kind of brain tumor (seven cases) and mouse cerebellum (one case) were all less than 1 ng/ml (Table 1).

      In the chromophobe adenoma, hGH levels in the medium decreased gradually during the culture. After three weeks, it was not detected in most cases. In this series of tissue culture, it might have been possible that high hGH levels were caused by the contamination of normal pituitary tissue which remained in the peripheral portion of the tumor. In most cases, histological examination of the tumor revealed that the compressed normal pituitary tissue was present in the peripheral portion of the adenoma.

      To investigate this possibility, both central and peripheral portions of the tumor were cultured separately in five chromophobe adenomas, case C1, C6, C7, C8, and C9. The peripheral portions yielded higher hGH level than those of the center. Even in the center of the tumor, however, hGH levels were more than 20 ng/ml in three adenomas and 4.1 and 3.5 ng/ml in the other two (Table 1, Fig. 9).

   b) acromegaly
      In all of four acromegalic patients, hGH levels remained high through longer periods of culture compared to chromophobe adenoma (Table 1). Two of them showed extremely high levels of hGH of above 880 ng/ml for more than a month.

2) prolactin
     High hPr levels of more than 50 ng/ml in the culture medium were detected in not only the Forbes-Albright syndrome, but also acromegaly or non-functioning tumor with hyperprolactinemia (Table 2).

Discussion

Electron microscopic studies of human pituitary adenoma were reported by Luse in 1962, Schelin in 1962, Fukumitsu and Kageyama in 1963, and other investigators. From these reports it has been well-known that round secretory granules of 300 μm in diameter were demonstrated invariably in both eosinophilic and basophilic adenomas and those of not only 100 but also 300 μm in most chromophobe adenomas.

Recently it has become possible to identify various hormones in pituitary cells with peroxidase-labeled antibody method. Using this method, Zimmerman et al. in 1974 described that hGH was located in the tumor cells in seven of eight acromegalic patients and also hPr in 10 of 12 pituitary adenomas with hyperprolactinemia. They also reported that tumor hGH was found in one patient with normal plasma hGH, while tumor hPr was found in one patient with normal plasma hPr.

On the other hand, quantitative assay of hormone in the culture medium of pituitary adenomas has been reported by Kohler.
Fig. 8. Electron micrograph, adenoma cells of acromegaly. Lower: Round secretory granules of 300 to 500 mµ in diameter diffusely in the cytoplasm. Upper: Electron micrograph of three week culture cells. Many secretory granules of 400 to 600 mµ in diameter, forming follicular structure with microvilli (upper right). × 6500.
Batzdorf4), and Teraoka34). Kohler et al. reported that hGH was detected in all five acromegalic patients and only one out of five chromophobe adenomas. Batzdorf cultured eight chromophobe adenomas and detected hGH in two cases.

In our study of 15 clinically non-functioning chromophobe adenomas, significant levels of hGH were demonstrated in all cultured media. With the Maximow double cover glass method, pituitary adenoma can be cultured with similar structure to that of the original tumor. This is important in studying the property of the tumor because changes in the nature of tumor cells had been reported in some culture methods1). Another advantage of this method is that the only small amounts of culture medium are required for the amount of explanted tissue. This makes it possible to detect even small amounts hormones in the medium.

In our series, hGH concentration in the medium was less than 5 ng/ml in seven of 15 chromophobe adenomas. It may arouse suspicion as to the accuracy of these values. However, GH sensitivity in our assay system was 1 ng/ml, and hGH levels in all adenomas were more than 1 ng/ml as compared with less than 1 ng/ml in all of the control groups. Moreover gradual decrease in hGH levels during the culture occurred in parallel with morphological disappearance of secretory granules. Thus, it seems sure that all the chromophobe adenomas in our series had the capacity to secrete growth hormone.

In pituitary adenomas of acromegalic patients, histological examination showed them to be either eosinophilic or chromophobe. Electron microscopic observation revealed that adenoma cells had secretory granules of 300 to 500 m\(\mu\) in diameter diffusely in the cytoplasm. With tissue culture, high hGH was measured in the medium.

Recently it has been recognized that hGH hypersecretion in acromegalic patients responds to various stimuli to the hypothalamus in some cases. A suppression of hGH levels following glucose ingestion6,8,22), and its rise subsequent to insulin hypoglycemia, arginine infusion6,22), and provocation with thyrotropin releasing hormone16) have been reported. From these clinical data, Sherman et al. suggested that pituitary tumors could result from chronic hyperstimulation through hypothalamic mechanisms33), and Daughaday et al. have hypothesized that a defect in hypothalamic regulation of GH secretion may underlie the development of acromegaly in some patients6).

One the other hand, Allen et al. reported two acromegalic cases in which remission with maintenance of normal endocrine function lasting one year was achieved by selective pituitary adenomectomy. They suggested that acromegaly might be due to autonomous pituitary tumor.

From experimental data of tissue culture, Kohler et al.21) described that adenoma cells from acromegaly seemed to retain such a differentiated function as producing a hormone in vitro, but normal pituitary cells were likely to exhibit dedifferentiation in the early stages of culture. Batzdorf et al.4) reported that adenomas from acromegaly synthesized and secreted GH independently from hypothalamic control.
In our study of tissue culture, gradual decrease of GH levels in chromophobe adenoma occurred in parallel with morphological disappearance of secretory granules. In acromegaly, all GH levels were over ten-fold as compared with those in chromophobe adenoma and remained high even after one-month culture. Many secretory granules were also observed in the cytoplasm at this stage. The fact that the capacity to secrete hGH remains longer than that of normal pituitary tissue or non-functioning tumor in vitro suggests that the tumor cells in acromegaly have the capacity to secrete hGH independently from hypothalamic control.

Excessive prolactin secretion is frequently encountered in patients with pituitary tumors. While some patients with excessive prolactin secretion show galactorrhea, often no manifestation are recognized clinically. In Forbes-Albright syndrome and also non-functioning tumor or acromegaly, secretion of prolactin as well as growth hormone was demonstrated in the culture medium. Electron microscopic study revealed big ovoid, ellipsoid, irregular secretory granules of 500 to 1000 µm in acromegalic patients, while granules of similar shape but of smaller size of 150 to 500 µm were noted in non-functioning tumor and Forbes-Albright syndrome. According to electron microscopic studies of human pituitary gland, lactotrophs were said to contain irregular or ovoid homogeneously dense granules as large as 400 to 800 µm. Our prolactin secreting adenoma cells are strikingly similar to the above descriptions of human lactotrophs. Thus prolactin secretion from tumor cells is probably attributed to these granules.

Acknowledgement

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References


