Quantitative Measurement of the Ability of Human Chromophobe Adenomas to Synthesize and Release Human Growth Hormone

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Summary

Two endocrine functions, HGH synthesis and release, were separately studied on human chromophobe adenomas, isolating the hormone with polyacrylamide gel disc electrophoresis after in vitro labelling of the hormone with $^{14}$C-leucine. The results of the experiments utilizing fresh adenoma tissues obtained from 8 cases at operation revealed that all of the adenomas synthesized and released HGH. Employing appropriate parameters, HGH synthesis and release functions of the tumor were estimated. HGH synthesis function was likely to be extremely low, but the release function rather high. HGH content in the adenoma tissues ranged from 0.04 to 2.56 ng/mg wet tissue, which was exceedingly smaller than the value of 8000 ng/mg wet tissue observed with the human anterior pituitary obtained at autopsy.

Key words; Chromophobe adenoma. Human growth hormone. HGH synthesis. HGH release

I. Introduction

Electron microscopic observation of the secretory granules in human chromophobe pituitary adenoma cells and demonstration of human growth hormone (HGH) and other anterior pituitary hormones in the culture medium of chromophobe adenoma have strongly suggested that tumor cells can produce a limited amount of hormones. These facts lead us to believe that the adenoma is qualitatively a functioning tumor. The purpose of this paper is to present the results of our study in which the ability of the tumor to synthesize and release HGH was quantitatively determined.

II. Materials and Methods

Fresh chromophobe adenoma tissue was obtained from nine patients of both sexes when the tumor was subcapsularly removed. Clinical examination before each operation showed signs and symptoms of pituitary hypofunctions and ocular symptoms such as atrophy of the optic nerves and defect of visual fields respectively (Table 1). Insulin-induced hypoglycemia provoked only a decreased response; the peak value of plasma HGH ranged from 2.1 to 7.8 ng/ml, the values considerably lower than in...
normal humans. Histological examination of the tumor specimens revealed that they were all chromophobe adenomas mixed with no normal pituitary tissue.

Anterior pituitaries, isolated 3 to 5 hr. after the death, were also utilized for HGH identification. Out of the nine autopsy cases, seven had tumors in the cerebral hemisphere and two acute traumatic subdural hematomas.

In the first step of the experiment, the position of HGH band was determined in a polyacrylamide gel column after electrophoresis. 5-15 mg tissue was taken from each anterior pituitary obtained at autopsy and from individual fresh specimen of the adenoma, and were homogenized with 0.2 ml of distilled water. Each homogenate was subjected to polyacrylamide gel disc electrophoresis after Jones et al. In order to locate protein bands, the gel column was stained with an amido black-7% acetic acid solution. The column, with which HGH content was to be measured, was stained lightly with a highly diluted dye solution (0.002 %) for less than 20 min. to avoid possible disturbance at radioimmunoassay. After staining, the ratio of the distance of each band to that of the dye front (Rf) and the ratio of the distance of the band to that of the hemoglobin band (Rf/refer) were determined. Thereafter the lightly stained bands were dissected, and proteins were eluted twice with a 0.5 ml phosphosaline buffer solution, pH 7.6 in glass homogenizers. After each elution, the gel homogenate was centrifuged at 2000 rpm for 5 min., and the supernatant fluid was subjected to the same treatment as mentioned above, and the radioactivity of each protein band was counted.

The ability of the adenoma tissue to synthesize and release hormones was determined by the following parameters, similar to those utilized for rat anterior pituitary: 

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\text{Hormone synthesis function: } \frac{t + m}{mg \text{ wet tissue}}, \\
\text{Hormone release function: } 100 \times \frac{m}{t + m},
\]

where \( t \) (cpm) is the radioactivity of newly synthesized hormone during the incubation and retained in the tissue, and \( m \) (cpm) is that of the hormone newly synthesized and released into the medium. The term \( 100 \times \frac{m}{t + m} \) is the percent release of the newly synthesized hormone. The hormone synthesis and release functions of the chromophobe adenoma were thus estimated separately.

III. Results

1. Identification of HGH band and quantification of the HGH content

1) Anterior pituitaries

A gel column deeply stained with 0.5% dye solution is shown in Figs. 1 and 2. Two bands, one thin and the other thick, appeared between hemoglobin and albumin bands, and were designated as \( U_1 \) and \( U_2 \) (Fig. 2). The HGH content estimated by radioimmunoassay was around 8000 ng/mg wet tissue for \( U_2 \) band, and no HGH was detectable in the other bands.
Thus, U2 was identified as the HGH band. Rf value of the HGH band was 0.67 in most cases, and Rf/refer was around 1.37 (Fig. 2). The Rf of U1 was 0.56 in many cases which coincided with the Rf of one main band obtained with a human prolactin sample, RES.

STD A prolactin, human pituitary 71/222, National Institute for Biological Standard and Control.

2) Chromophobe adenomas

In all the gel columns, HGH band with Rf value of approximately 0.67 was clearly detectable even when lightly stained. The HGH content of this band was measured in all nine cases (Fig. 3). Three cases gave values below 0.12 ng/mg wet tissue, while the other six cases showed values ranging from 0.48 to 2.56 ng/mg wet tissue. These results clearly indicated that HGH existed in the chromophobe adenomas and was isolated as a clear band in the gel column.

In three cases of chromophobe adenomas (Cases 4, 6, 9), U1 bands with Rf values of around 0.56 were detectable when gel columns were lightly stained. Among these three cases, Case 9 clinically manifested amenorrhea-galactorrhea syndrome and had an elevated plasma prolactin level.

II. HGH synthesis and release functions of chromophobe adenomas

In eight cases of chromophobe adenoma (Case 5 was excluded because the incubation was delayed), two distinct peaks of radioactivity were observed in the gel column. Fig. 4 shows the radioactivity distribution in the gel column prepared with the tumor from Case 9. These peaks correspond to U1 and HGH bands. Incorporated radioactivity into HGH from the adenoma tissue (t) and incubation medium (m) is listed in Table 2. As shown in this table, HGH synthesis function, (t + m)/mg wet tissue, was high in Cases 1, 4, 6 and 9 ranging from 102 to 220 cpm, and low in Cases 2, 3, 7 and 8 ranging from 70 to 88 cpm.

HGH release function, 100 × m/(t + m), was
35.2% in average. Cases 1, 2 and 3, in which large cyst formations were found within the tumors, showed a low release function (less than 25%).

As mentioned above, U₁ band was observed in 3 cases out of 8. In these cases, t value ranged from 116 to 150 cpm/mg wet tissue, and m value from 53 to 82 cpm/mg wet tissue. Functional parameters of the synthesis and release functions ranged from 168 to 224 cpm/mg wet tissue and from 31 to 38%, respectively.

IV. Discussion

It was formerly believed that chromophobe adenoma of the human pituitary was non-functioning, mainly because patients with the tumor usually manifested clinical signs and symptoms of panhypopituitarism. Later, however, it was suggested that chromophobe adenomas were functioning based on the electron microscopic demonstration of the secretory granules in the tumor cells. In recent studies on cultured chromophobe adenoma cells, HGH, LH and TSH were found in the medium. Kageyama et al. confirmed that chromophobe adenomas produce HGH, though in exceedingly small quantities, employing both electron microscopy and tissue culture. Thus, it seems now to be an undeniable fact that the chromophobe adenoma is qualitatively a functioning tumor. But further confirmation and quantitative determination of hormone synthesis and release functions of the tumor are considered necessary.

A method has been recently established, by which two distinct functions, i.e. synthesis and release of some pituitary hormones, can be determined separately, and reasonable functional parameters representing these two functions have been proposed. The method and parameters, originally applied to rat anterior pituitary, made our analytical study on the functions of HGH synthesis and release by human chromophobe adenomas possible. The present report is the first to demonstrate the HGH synthesis and release functions of the chromophobe adenoma quantitatively.

The position of HGH in polyacrylamide gel column had to be located first, since the position was different from that of rat GH. Under our electrophoresis conditions, Rf and Rf/refer values of HGH were estimated to be approximately 0.67 and 1.37, respectively.

Four cases (Cases 1, 4, 6, 9) showed higher HGH synthesis function than the other cases. Case 4, showing the highest function, was clinically a so-called malignant adenoma, which extended into the left middle fossa destroying the entire sella turcica and the left sphenoidal ridge. Three cases with cyst formation within the tumor (Cases 1, 2, 3) showed low release function. Generally, the synthesis and release functions were not parallel to each other. Further cases have to be examined to correlate the functions with clinical and pathomorphological features.

It was not possible for us to compare HGH synthesis and release functions of chromophobe
adenoma with those of normal human pituitary, since there was no opportunity to utilize normal tissue in a fresh state. The present study showed extremely lower GH synthesis function and higher release function than those observed with normal adult rats. Whether or not these differences can be ascribed to difference in species is unknown at present, but it strongly suggests a very low GH synthesis function of the human chromophobe adenomas. Extremely low HGH content of the tumor supports the assumption. Though HGH release function is high in our cases, the low synthesis function and low HGH content of the tumor would permit release of only an extremely small amount of HGH.

In order to increase the validity of our experiment, incubation of the adenoma tissue was begun immediately after they were removed. The tumor tissue from Case 5 was not used for studying synthesis and release functions, because the incubation was started 30 min. after removal. The same reason applies as to why the anterior pituitaries isolated at autopsy were not utilized in measuring pituitary functions.

Histological examination showed no possibility of adenoma tissue being mixed with normal anterior pituitary tissue.

In the present experiment, HGH release function was measured only for labelled hormone. However, there is evidence indicating that newly synthesized, labelled hormones and stored hormones (GH and prolactin) are released at the same rate in the rat (Yamamoto et al., unpublished data).

The present study suggested that chromophobe adenomas possess prolactin synthesis and release functions. A case (Case 9) with amenorrhea-galactorrhea syndrome showed the highest incorporation of $^{14}$C-leucine into U$_1$ band among the cases studied, indicating the highest synthesis function. This band showed an Rf value of one main band of a standard prolactin sample, which showed several separated bands. An Rf value of 0.55, which is close to ours, was reported for human prolactin. However, the presence of prolactin in this and the other bands should be further studied in order to determine the position of prolactin in the gel column. This line of study is now being carried out in our laboratory.

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References