# THE EFFECTS OF GROWTH HORMONE-RELEASING HORMONE(GH-RH) and THYROXINE ON THE SYNTHESIS AND RELEASE OF GH IN A CLONAL STRAIN OF RAT PITUITARY TUMOR CELLS

Hiroshi Ishikawa,Takahisa Nagayama,Chieko Kato,and Masuo Takahashi

Department of Anatomy, Tohoku University, School of Medicine Seiryo-cho, Sendai 980, Japan

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Summary: The GH 1 clonal strain was cultured with serum free-NCTC-109 medium(control), GH-RH-NCTC-109 medium, l-thyroxine-NCTC-109 medium, and GH-RH-thyroxine-NCTC-109 medium for 8 days each. Results showed a slight tendency toward increase of GH synthesis in the GH-RH medium, but more of a tendency toward release than synthesis of GH. However, when physiological concentrations of l-thyroxine were added to the GH clonal cells, synthesis was increased to about three times that of the control, although GH release was not enhanced. The increase of GH synthesis was about eight times that of the control when l-thyroxine was added to the GH-RH medium.

It has been demonstrated that GH synthesis and release are stimulated by the addition of GH-RH in vivo and in vitro (1,2,3,4,5). It has also been demonstrated that the amounts of TSH in the anterior pituitary and in the blood are increased after thyroidectomy, but the GH content in the pituitary is markedly decreased(6,7) even though the GH-RH content in the hypothalamus is almost unaltered after thyroidectomy. The acidophils in the adenohypophysis also disappear with the decrease of GH content after thyroidectomy. But after administration of thyroxine or iodine to the thyroidectomized rats, GH content and the number of acidophils in the pituitary recover immediately. These results clearly suggest that GH-RH and thyroxine have a stimulating effect on GH synthesis, and that iodine also stimulates (whether directly or indirectly is unclear) the production of GH.

Thyroidectomy data seems to suggest that the stimulating effect of GH-RH on GH synthesis does not appear in the absence of thyroxine: although there is no decrease in GH-RH content in the hypothalamus after thyroidectomy, there is a marked decrease in GH content in the pituitary, a decrease not caused by administration of thyroxine.

It is necessary to use a homogenous cell population (for example, GH clonal cells) to isolate the effect of thyroxine on the synthesis of GH. We reported earlier on the experiment utilizing a GH clonal strain from normal rat pituitaries: thyroxine stimulated both the production and cytodifferentiation of GH-secreting cells(8).

For this report, we hoped to study the effects of GH-RH and thyroxine on the GH synthesis of GH-secreting cells, but the GH clonal strain from normal rat pituitaries could not be cultured in a serum-free(thyroxine and iodine-free)medium for a long period of time. If we had used a serum-supplemented medium, we could not have excluded all the thyroxine and iodine from the medium. Therefore, in order to be able to differentiate the actions of GH-RH and thyroxine on GH synthesis, we decided to use a GH 1 clonal strain from rat pituitary tumor cells, as this strain can be cultured over a long period of time in a serum-free medium.

Materials and Methods: For this study, we utilized the GH 1 clonal strain of rat pituitary tumor cells isolated by Yasumura and colleagues(9). These cells retain the organ-specific capacity of production of GH during cell propagation. These GH 1 cells had been cultured in a medium of 85% Ham's F10, 2.5% foetal calf serum and 12.5% horse serum, in 75ml plastic flasks(Falcon #3024), in a humidified atmosphere of

5% $CO_2$, 5% $O_2$ and 90% air, at 37°C. For seven days prior to the experiment, however, they were cultured only with serum-free NCTC-109 medium(10) supplemented with BSA(14).

The GH content in the cells and in the media were assayed using the double antibody radioimmunoassay method of Ohtsuka,et al.(11). The medium was renewed every two days. GH content in the cells was assayed after 8 days' culture in each medium, and GH content in the media used in the last two days was assayed. Synthetic GH-RH was donated by Tanabe Seiyaku Co.LTD.. The GH-RH-medium contained 0.30 μg of synthetic GH-RH per ml of NCTC-109 medium. The sodium salt of l-thyroxine was obtained from Teikokuzoki Co.LTD.. One mg of l-thyroxine was dissolved in 1 ml of 0.05 NaOH, then diluted in 2,000 ml of NCTC-109 medium. The GH-RH-thyroxine medium contained both GH-RH and thyroxine, in the concentrations outlined above, per 1 ml of NCTC-109 medium.

Results: In the cells(assayed after 8 days' culture) and medium(that used in the final two days) of the control culture(BSA-supplemented NCTC-109 medium), there was found 32μg of GH in the cells and 0.97 μg of GH in the medium per 1 mg of cell protein. In the GH-RH medium, there was 38 μg of GH in the cells and 2.15 μg of GH in the medium. In the thyroxine medium, there was 96 μg of GH in the cells and 1.13 μg of GH in the medium. There was a great increase, however, in GH content in both the cells and the medium in the GH-RH-thyroxine medium: 249 μg of GH in the cells and 3.3 μg of GH in the medium(Table 1). These results suggest that thyroxine is necessary for the synthesis of GH, and that GH-RH works to further stimulate the effect of thyroxine on GH synthesis. Thyroxine seems not to be a release-stimulator of GH, as is GH-RH, but is rather a synthesis-stimulator of GH.

Table 1. Comparison of amount of GH in the cells and in the media of GH 1 cells cultured with control-, GH-RH-, thyroxine-, and GH-RH-thyroxine-medium for 8 days.*

| Medium condition | No. of flasks | GH content (μg/mg cell protein) | |
|---|---|---|---|
| | | Cells | Media*** |
| Control | 8 | 32 ± 5.3** | 0.97 ± 0.14 |
| GH-RH | 6 | 38 ± 4.6 | 2.15 ± 0.22 |
| Thyroxine | 6 | 96 ± 11.8 | 1.13 ± 0.77 |
| GH-RH-thyroxine | 6 | 249 ± 33.1 | 3.39 ± 0.86 |

* The cells were usually cultured in Ham's F10 medium 85%, foetal calf serum 2.5%, and horse serum 12.5% containing medium. The cells were cultured in each experimental medium for 8 days after being preincubated with serum-free NCTC-109 medium for 7 days.
** Mean ± S.E.
*** The assay medium was that used in the last 2 days of culturing.

Discussion: After thyroidectomy of a rat, the GH cells in the anterior pituitary disappear in about 14 days, but upon administration of iodine or thyroxine, the cells recover in a few days(6,7). Suzuki and Ishibashi(13) demonstrated that thyroxine stimulates the synthesis of GH and prolactin in the adenohypophysis of a thyroidectomized rat in vivo.

Recently, Ishikawa and Nagayama(12) demonstrated that the GH clonal cells(strain) of normal rat pituitaries differentiated morphologically and functionally after being cultured in a thyroxine supplemented medium. The cells enlarged gradually showing an increase in the mitochondria and the rough-ER, and secretory granules about 300-350 mμ in diameter appeared.

Because we cultured GH 1 clonal cells in a thyroxine medium, and did not culture them in an iodine medium, it is still unknown whether or not iodine will stimulate the growth if GH cells and synthesis of GH in vitro, as it did in vivo.

Schofield(15) and Wilber et al.(16) demonstrated that GH release is stimulated by GH-RH through the action of cyclic-AMP(c-AMP). Recently, there have been several reports dealing with thyroxine-receptor in the cells. DeGroot et al. (17) found the nuclear triiodothyronin-binding protein. Safi et al.(18) succeeded in separating the soluble fraction that contains thyroxine receptor from porcine anterior pituitary. Samuels et al.(19), using cultured GH 1 cells, reported the existence of thyroxine receptor in isolated nuclei obtained from those cells. These reports suggest that in GH cells, the receptors for GH-RH and thyroxine differ.

Thus in the present study, in order to clarify the difference in the effects of GH-RH and thyroxine on the synthesis and release of GH, GH 1 clonal cells were cultured separately in GH-RH, thyroxine, and GH-RH-thyroxine media. There have been many reports up to this time of GH-RH stimulation of synthesis and release of GH both in vivo and in vitro, but all these studies cite data based on the presence of serum. The present study has been conducted under serum-free conditions, permitting the discovery of the true effects on GH cells of GH-RH and thyroxine in isolation.

It has thus become clear that GH-RH stimulates the release rather than the synthesis of GH, while thyroxine acts to stimulate the synthesis of GH. GH-RH does not appear to stimulate the differentiation or maturation of GH cells; one would expect much greater synthesis and release of GH in a culture of GH cells in thyroxine to which GH-RH had been added, than in a culture of GH cells in GH-RH alone. That is, if GH-RH is caused to act upon mature GH cells, the simultaneous synthesis and release of GH that occur may be due

to the mediating action of c-AMP. The GH cells used in this experiment were not morphologically mature, however, and perhaps for that reason GH-RH did not greatly stimulate GH synthesis. Thyroxine seems to stimulate GH synthesis, however, through stimulation of cytodifferentiation or maturation of GH cells. DeGroot et al.(20) reported on the existence of triiodothyronine-binding protein bound to chromatin in the nucleus.

These various data and speculations bring up to four questions deserving of further research:

1) Does DNA amplication participate in the effect of thyroxine on the growth of GH cells and GH synthesis?

2) Is there a difference in the effect of GH-RH on GH synthesis and release in mature and immature GH cells?

3) Does c-AMP mediate in the action of thyroxine?

4) If c-AMP mediate in the action of thyroxine, in a culture of GH cells in GH-RH, c-AMP is greatly increased due to the action of the GH-RH, but why doesn't this increased c-AMP act on nuclear substances and stimulate GH synthesis, as occurs in cultures of immature GH cells in thyroxine?

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