Growth Hormone Releasing Factor Activity in the Stalk-Median Eminence and Plasma Growth Hormone Response to the Ether-Laparotomy Stress in the Rat

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Synopsis

The present study was performed to aim to the development of the in vitro assay method for growth hormone releasing factor (GH-RF) activity in the rat stalk-median eminence (SME), and to reveal the relationship between GH-RF activity in SME and plasma GH levels under ether-laparotomy stress in the rat.

SME extract was incubated with a total of 8 quartered anterior pituitaries. GH released into the medium was measured by polyacrylamide gel disc electrophoresis-densitometry system, and was used as an index of GH-RF activity. Plasma GH levels were estimated by the Rat GH radioimmunoassay kit (NIAMD).

Under manual handling, no significant change occurred in the levels of GH-RF activity in SME and in plasma GH levels. The ether-laparotomy (EL) stress caused a significant rise in GH-RF activity in SME at 15 min and reciprocally a significant decrease in plasma GH at 5 min and 15 min after starting of EL stress. Namely, there was an inverse relationship between the response of GH-RF activity in SME and that of plasma GH under EL stress. These findings may suggest that the EL stress depress the GH secretion via the hypothalamus, and diminution of GH secretion could be attributed to inhibition of GH-RF release or increased secretion of an inhibitory factor.

The evidence for the existence of growth hormone releasing factor (GH-RF) in the hypothalamic extracts of animals of different species has been reported during the past decade, and at this time, it has seemed reasonable to conclude that the hypothalamic control of GH secretion, like that of the other pituitary hormones is mediated by a hypophysiotropic releasing factor (Schally et al., 1968; McCann and Poter 1969).

Numerous workers have tested hypothalamic extracts for GH-RF activity in various assay systems (Schalch et al., 1968; Reichlin and Schalch, 1969; Schally et al., 1970).

The present paper aims to the development of assay methods for rat hypothalamic GH-RF activity that will be sensitive enough to detect quantitative changes in hypothalamic content of GH-RF, and reveals the relationship between hypothalamic GH-RF activity and plasma GH levels under ether-laparotomy stress in rats.

Materials and Methods

1) In vitro assay for growth hormone releasing factor (GH-RF) activity

Preparation of pituitary gland homogenate and stalk-median eminence (SME) extracts

Male Wistar rats weighing from 180 to 200 g were used for all experiments. The rats were killed by decapitation and SME and/or pituitaries were removed. The anterior lobes of the pituitary glands were dissected from the posterior lobes and were ground in a glass homogenizer using 0.05 M phosphate buffer.
buffer, pH 7.5. The homogenate of anterior pituitaries was centrifuged at 2,500 rpm for 10 min and the supernatants were used. The SME were homogenized in ice cold 0.1 N HCl (0.25 ml per SME). The homogenate was centrifuged at 3,000 rpm for 20 min at 4°C. Just prior to use, the supernatants were placed in medium 199 and the pH was adjusted to 7.4 by adding 1 N NaHCO₃.

**Disc electrophoresis**

The standard analytical polyacrylamide disc electrophoresis procedure of Davis (1964) was used. The concentration of acrylamide used for the separating gel was 7.5%. Sample gel (0.4 ml) containing 0.2 ml of the incubation medium or the reference standard solution was added to each electrophoretic column and a current of 5 milliamperes per column was applied at pH 9.5 for ca 50 min. After completion of electrophoretic separation, the column was stained in 1% amido black in 7% acetic acid solution. The optical density of GH band was measured with a densitometer (Fuji-Riken, Model FD-A IV). When the electrophoresis was carried out for the solution involving 6.5-100 µg of Rat GH (NIAMD-Rat GH-B-2), it was demonstrated that the linear relationship existed between the dose of Rat GH and the optical density of the band obtained from electrophoresis.

**Rat growth hormone (GH) radioimmunoassay**

GH was assayed by the Rat GH radioimmunoassay kit which was the kind gift from Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases.

**Identification of Rat GH band on the gels**

i) **Radioisotope analysis**

¹²⁵I-labeled Rat GH was applied on the disc electrophoresis. Gels were placed onto Parafilm sheets and were sliced 3 mm in length. Then, radioisotope counting was performed on transverse gel slices. On the other hand, the gel was sectioned longitudinally and was covered with Saran wrap, and then it was exposed to X-ray film for 30 days.

ii) **Radioimmunoassay for transverse gel slices**

Transverse gel slices were homogenated in 0.05 M phosphate buffer, pH 7.5. Aliquots of supernatants were subjected to radioimmunoassay.

**Incubation procedure of anterior pituitary tissue in vitro**

The anterior pituitary of the donor rat was cut into 4 quarters, and a total of 8 quarters (2 anterior pituitaries equivalent) were placed in each flask. The pituitaries were preincubated in medium 199 for 30 min at 37°C and the medium was discarded. One ml of fresh medium 199 containing the neutralized acid extract of SME (2 SME equivalent) was then rapidly added to flasks, and then final incubation was carried out for 30 min at 37°C. GH released into the medium was measured by above-described disc electrophoresis-densitometry, and was used as an index of GH-RF activity.

**2) Ether-laparotomy stress**

Rats were fed the pellet diet and tap water ad lib., and then they were fasted overnight before the experiments. The test animal was placed in a jar containing an ether/air admixture for 2 min, and then has undergone the laparotomy. During experiments, continuous ether anesthesia was maintained by dropping ether onto a cone placed loosely over the head after induction in the ether jar. Animals were decapitated at different time intervals (Fig. 4), and trunk blood was collected in heparinized test tubes and the SME were removed. The blood was centrifuged immediately, and the plasma was separated and stored at −20°C until GH was assayed. The SME were homogenated and assayed GH-RF activity as described above.

**Results**

1) **In vitro assay for GH-RF activity**

Disc electrophoretic pattern of rat anterior pituitary homogenate and incubation medium were quite similar (Fig. 1 A-a, A-b). The major band obtained with pituitary homogenate and incubation medium showed the same mobility as that of the standard Rat GH preparation (NIAMD-Rat GH-B-2) (Fig. 1 A-c).

The Rat GH band was located on the polyacrylamide gels by autoradiography (Fig. 1-B), radioimmunoassay (Fig. 1-C) and radioisotope analysis (Fig. 1-D). These GH bands or peaks showed the same mobility, and relative electrophoretic mobility (Rf) values derived from the disc electrophoresis (7.5% gel, pH 9.5) were 0.452 ± 0.002 (Mean ± SE).

Various samples (incubation media) were analyzed by disc electrophoresis and radioimmunoassay. There was an exceptionally close correlation between the values of both methods: $y = +0.877$, $P < 0.001$, $y = 0.83x + 38.7$, where $y$ was the GH levels...
Fig. 1. Identification of rat growth hormone band in polyacrylamide gel columns. (GH: growth hormone, Al: albumin, P: prolactin, bf: buffer front) A: Disc electrophoretic pattern (stained with amido black) of anterior pituitary homogenate, incubation medium, and standard rat growth hormone preparation (NIAMD-Rat GH-B-2). B: Autoradiography of longitudinal sectioned gel. C: Radioimmunoassay of rat growth hormone levels in transverse gel slices. D: Radioactivities of transverse gel slices.

Table 1. Effect of graded doses of anterior pituitary on GH release during 30 min incubation.

<table>
<thead>
<tr>
<th>No. of anterior pituitaries</th>
<th>Medium GH (optical density unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
</tr>
<tr>
<td>1</td>
<td>14.8 ± 1.4**</td>
</tr>
<tr>
<td>2</td>
<td>26.3 ± 2.0</td>
</tr>
<tr>
<td>3</td>
<td>44.0 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>rat SME*</td>
</tr>
<tr>
<td>1</td>
<td>21.3 ± 3.4</td>
</tr>
<tr>
<td>2</td>
<td>53.5 ± 2.1</td>
</tr>
<tr>
<td>3</td>
<td>63.3 ± 2.5</td>
</tr>
</tbody>
</table>

* SME, rat, equivalent to 2 hypothalami added. ** Mean ± SE of 4 flasks containing 8 quarters of pituitaries each.

gives a good estimate of its immunological activity. Hence, disc electrophoresis is a very convenient method for quantitative analysis of GH in the anterior pituitaries of rats and in the incubation medium.

In the incubation of anterior pituitary tissue as a GH-RF activity assay, effect of graded doses of anterior pituitary on GH release during 30 min incubation is shown in Table 1. And, the dose-dependent effect of SME preparation (NIAMD-Rat HE-RP-1) is
Fig. 3. Logarithmic dose-response curve showing pituitary GH release as a response to SME extract (NIAMD-Rat HE-RP-1). (Optical density units are in proportion to the area of GH peak on the densitometer recording).

shown in Figure 3. The log-dose response was linear between the levels of 0.1 and 4 SME equivalents.

2) Effect of ether-laparotomy (EL) stress on plasma GH and the GH-RF activity content of SME in rats (Fig. 4)

The mean plasma GH level decreased markedly from zero time value of 107.4 ± 18.0 ng/ml to 39.5 ± 11.3 ng/ml (P < 0.01) and 60.8 ± 6.8 ng/ml (P < 0.05) at 5 and 15 min after starting of EL stress, and reciprocally the GH-RF activity content of SME increased significantly (P < 0.05) at 15 min after. Namely, GH secretion in plasma showed a reciprocal relationship to the GH-RF activity content of SME. On the other hand, no significant difference was noted in plasma GH levels and the GH-RF activity content of SME, under the manual handling at zero time.

Discussion

In the first place, the quantitative analysis of Rat GH was investigated by disc electrophoresis. The major band obtained with pituitary homogenate showed the same mobility as that of standard Rat GH (NIAMD-Rat GH-B-2), which was in quite agreement with the result of Lewis et al. (1965) and Yanai et al. (1968). The present study reveals that the major band obtained with the medium after incubation of anterior pituitary
tissue also showed the same mobility. Furthermore, in order to confirm that this major band of pituitary homogenate or incubation medium was GH band, the autoradiography, radioimmunoassay and radioisotope analysis were performed. By these experiments, it was also improved that this major band of the polyacrylamide gel was GH band.

Yanai et al. (1968) and Daughaday et al. (1970) reported that the polyacrylamide gel disc electrophoresis-densitometry system could be employed at least for quantitative analysis of rat or mouse anterior pituitary level of GH. The present study demonstrated that there was an exceptionally close correlation between the GH levels of incubation medium obtained from the radioimmunoassay and that from disc electrophoresis-densitometry system (Fig. 2). This fact reveals that this system could be also employed for quantitative analysis of medium after incubation of anterior pituitary tissue in vitro. These observations suggest that the in vitro anterior pituitary tissue incubation-disc electrophoresis-densitometry system can be employed to assay the GH-RF activity. This system is relatively simple to perform and has been found to be suitable for measuring quantitative change in hypothalamic GH-RF content produced by different physiological states.

It has been reported that a variety of stress stimuli depress plasma GH in the rat in contrast to their stimulatory effect in other species (Schalch and Reichlin, 1968; Garcia et al., 1968; Takahashi et al., 1971; Kokka et al., 1972). However, the evidence for hypothalamic regulation of GH secretion is generally accepted at the present time (Schally et al., 1968; McCann et al., 1969). So, it appeared worthwhile to study whether the decreased secretion of GH during stress stimuli involved decreased content of hypothalamic GH-RF activity. Simultaneous determinations of hypothalamic GH-RF activity and plasma GH levels in the rat clearly demonstrated that depletion of plasma GH levels caused by EL stress was accompanied by increasing of GH-RF activity of SME (Fig. 4). These observations may suggest that the non-specific stimulus as EL stress depresses the GH secretion via the hypothalamus, and diminution of GH secretion could be attributed to inhibition of GH-RF release or increased secretion of an inhibitory factor. No ready explanation about slight discrepancy in the timing between the peak of GH-RF activity in SME and the nadir of the plasma GH level is forthcoming, and considerable work will be required to explain this discrepancy.

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References


