Effects of ovine corticotrophin-releasing factor and hydrocortisone on growth hormone secretion by pituitary adenoma cells of acromegaly in culture

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Abstract. In an attempt to test the hypothesis that pituitary adenomas of acromegaly may possess altered cellular membrane receptors, the response of growth hormone (GH) secretion to ovine corticotrophin-releasing factor (CRF) in cultured adenoma cells of acromegaly was studied. In three out of seven experiments using different pituitary adenoma cells in culture, nanomolar concentrations of CRF caused a significant increase in GH release. The CRF-induced GH release was reproducible and a dose-response relationship was observed between the CRF concentrations and the amounts of GH released into the incubation media. Hydrocortisone, at a concentration of 1 \( \mu \text{M} \), on the other hand, resulted in a significant decrease in GH secretion in four out of five experiments. When adenoma cells were co-incubated with CRF and 1 \( \mu \text{M} \) hydrocortisone, CRF-induced GH release was partially overcome. In one experiment, the inhibitory effect of hydrocortisone was reversed by co-incubation with CRF, although CRF alone was ineffective in the stimulation of GH. These results suggest that CRF may stimulate GH release in some, though not all, patients with acromegaly, and that glucocorticoids may block this effect of CRF acting directly on the pituitary adenoma cells of acromegaly.

Anomalous or paradoxical responses of growth hormone (GH) secretion to various stimuli including dopaminergic agonists and thyrotrophin-releasing hormone (TRH) in acromegalic patients have been well documented. We have recently demonstrated that no qualitative difference in GH response to dopaminergic agonists exists between pituitary adenoma cells of acromegaly and normal pituitary cells in culture (Ishibashi & Yamaji 1984). The anomalous response to dopaminergic agonists may be ascribed, therefore, to an abnormality in the regulatory system of GH secretion in the hypothalamus, which may be responsible for the pathogenesis of acromegaly, or alternatively, which may have resulted secondarily from the overproduction of the hormone by pituitary tumours. The stimulatory effect of TRH on GH secretion in acromegalic patients, on the other hand, may reflect a possible alteration in cellular membrane receptors of pituitary adenoma, since TRH acts on adenoma cells of acromegaly to induce GH secretion (Ishibashi & Yamaji 1978, 1984; Adams et al. 1979) while such an effect of TRH could not be demonstrated in normal human somatotrophs in vitro (Ishibashi & Yamaji 1984).

Recently, corticotrophin-releasing factor (CRF) has been isolated from ovine hypothalamic extracts and its primary structure determined (Vale et al. 1981; Spiess et al. 1981). Synthetic ovine CRF is shown to be a potent and specific secretagogue for ACTH both in vivo (Rivier et al. 1982) and in vitro (Vale et al. 1981). The present study was undertaken to examine whether CRF, as TRH and luteinizing hormone-releasing hormone (LRH) (Rubin et al. 1973; Faglia et al. 1973), may paradoxically trigger GH release in acromegaly using pituitary adenoma cells in culture.
Materials and Methods

Subjects

Seven patients with acromegaly, 4 men and 3 women, were studied. The age of the patients ranged from 30 to 47 years (mean 38 years). They exhibited characteristic physical features of acromegaly, elevated baseline plasma GH concentrations (7–71 ng/ml, mean 30 ng/ml), and failure of plasma GH levels to suppress during an oral glucose tolerance test.

Monolayer culture of pituitary adenoma cells

Pituitary adenoma tissues obtained by transphenoidal adenomectomy were cultured in monolayer. Light microscopy revealed that six (Nos. 1–6) of them were eosinophilic, while the remaining one (No. 7) was chromophobic. The method for the monolayer culture of pituitary cells has been previously described in detail (Ishibashi & Yamaji 1981, 1984). In brief, pituitary adenomas were cut into small pieces, dispersed by incubation with trypsin-collagenase solution at 37°C, planted in plastic Petri dishes (35 × 10 mm, Corning Glass Works, Corning, NY) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The culture medium consisted of Eagle’s Minimum Essential Medium in Earle’s solution including 10% foetal calf serum, 100 U/ml penicillin and 10 μg/ml streptomycin sulphate. When cells were incubated with labelled leucine and harvested after increasing periods of time, incorporation of radioactivity into immunoprecipitable GH in cell extracts proceeded linearly for the entire 48 h incubation (Ishibashi & Yamaji 1984). Secretion of GH from adenoma cells in culture was well maintained as long as 1 month by changing culture medium at 2–4 days intervals (Ishibashi & Yamaji 1984), although a gradual decrease in GH release was seen when the culture was continued. These results suggest that de novo synthesis as well as secretion of GH is actively taking place in monolayer culture under the present experimental conditions.

Incubation of cells with CRF or hydrocortisone

Incubation studies were started after the cells formed a monolayer. Individual cultures were randomly allocated for each experiment. Four or more cultures were used for the control and variables, and run simultaneously.

On the day of experiment, the medium was replaced by 2 ml of Eagle’s Minimum Essential Medium in Earle’s solution containing 0.5% human serum albumin instead of foetal calf serum. Cells were incubated for 1 h at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The medium was then removed and cells were further incubated for 2 h in 2 ml of fresh medium with or without test materials. Synthetic ovine CRF was purchased from the Protein Research Foundation, Osaka, Japan and initially dissolved in sterile 0.9% saline, which was serially diluted to an appropriate concentration with the incubation medium immediately before each experiment. Hydrocortisone hemisuccinate was obtained from Japan Upjohn Co., Tokyo, Japan, dissolved in 0.9% saline and diluted with the incubation medium. Control dishes received vehicle alone. After incubation, the medium was centrifuged at 150 g for 10 min and the supernate was stored at −20°C until assayed.

Radioimmunoassay

GH concentrations in the medium of both pre-incubation and experimental incubation were determined by a radioimmunoassay, as previously described in detail (Ishibashi et al. 1977; Ishibashi & Yamaji 1978, 1984). Immunological materials for the radioimmunoassay were kindly donated by NIADDK and the National Pituitary Agency, US Public Health Service. The coefficient of variation averaged 5.3% for intra-assay error and 9.6% for inter-assay error. Results are expressed as the percentage of hormone secreted in the experimental incubation compared with that secreted during the pre-incubation for individual cultures. For comparison, the mean values obtained in the control study were designated as 100%.

Statistical analysis

Values in figures and text are the mean ± SEM unless otherwise specified. The significance of difference was calculated using Student’s t-test and analysis of variance.

Results

Dispersed cells of pituitary adenomas of acromegaly formed a monolayer attaching to the culture dishes within the first 48–72 h. The plating efficiencies of cells averaged 29%. Pituitary adenoma cells in monolayer culture actively secreted GH. The amounts of GH accumulated in the media during the pre-incubation ranged from 34.2 ± 2.6 (mean ± SEM, n = 21) (No. 5 in Fig. 1) to 5754 ± 197 ng/h (n = 34) (No. 2) per dish, while GH secretory rates during the experimental incubation in the control study ranged from 98 to 177% (111 ± 7.4, mean ± SEM, n = 11) of those during the pre-incubation.

In Fig. 1 is shown the effect of synthetic ovine CRF on GH secretion by pituitary adenoma cells from seven different acromegalic patients. In three experiments, the addition of ovine CRF to the incubation media significantly stimulated GH release (Nos. 1, 2 and 7). The minimum effective dose of ovine CRF required for a significant increase of GH secretion was nanomolar concentrations and a dose-response relationship was observed between the CRF concentrations and the
cells from acromegalic patients were co-incubated with ovine CRF and hydrocortisone (Fig. 2). In the adenoma cells Nos. 1 and 2, ovine CRF again increased GH secretion rate indicating that this effect of CRF is reproducible. When the adenoma cells were exposed to hydrocortisone at a concentration of 1 µM, GH accumulated in the incubation media was significantly reduced. Co-incubation of ovine CRF with hydrocortisone resulted in a significant inhibition of ovine CRF-stimulated GH release by adenoma cells, although the effect of 100 nM CRF was not completely abolished by 1 µM hydrocortisone. In two experiments using adenoma cells No. 3 and 4, CRF at a concentration of 100 nM did not show any stimulatory effect on GH secretion, which is consistent with the results illustrated in Fig. 1. The addition of hydrocortisone at a concentration of 1 µM, on the other hand, resulted in a significant inhibition of GH release. In one of these two experiments (No. 4), the inhibitory effect of hydrocortisone was reversed by co-incubation with CRF, although CRF alone was ineffective in the stimulation of GH secretion. In the remaining one experiment, neither CRF nor hydrocortisone produced any effect on GH secretion (No. 5 in Fig. 2).

Discussion

The foregoing results show that some, though not all, adenoma cells of acromegaly respond to ovine CRF to secrete GH in vitro. The response was reproducible and a dose-response relationship was demonstrated between the CRF concentrations and the amounts of GH secreted into the media suggesting that the stimulatory effect on GH secretion was occasioned by a specific action of ovine CRF. Ovine CRF has been shown to be a potent and specific secretagogue for ACTH in man (Orth et al. 1983) as well as in experimental animals (Rivier et al. 1982). No increase in the circulating levels of GH was observed in normal individuals after the injection of CRF even when a dose as high as 30 µg/kg body weight was employed (Orth et al. 1983). In view of this finding, the result of the present study may be interpreted by a possible alteration in cellular membrane receptors of adenoma cells of acromegaly. The frequency of this anomalous GH response to CRF in acromegalic patients is obscure. In vivo GH response to ovine CRF should be...
Effects of ovine CRF and hydrocortisone (H) alone or in combination on GH secretion by cultured adenoma cells of acromegaly. Results are expressed as indicated in the legend to Fig. 1. The number of adenoma cells coincides with that in Fig. 1. Secretory rates of GH during pre-incubation were 77.0 ± 6.0 (mean ± SEM, n = 33) (No. 1), 3500 ± 72.4 (n = 34) (No. 2), 1204 ± 191 (n = 30) (No. 3), 122 ± 13.6 (n = 20) (No. 4), 34.2 ± 2.6 ng/h/dish (n = 21) (No. 5), respectively. Results are the mean ± SEM. * P < 0.05 vs control; ** P < 0.01 vs control.

carefully examined in many acromegalic patients to answer this question.

The administration of glucocorticoids has been repeatedly shown to reduce or abolish the GH release to insulin-induced hypoglycaemia (Frantz & Rabkin 1964; Hartog et al. 1964). The attenuation of the basal GH secretion rate was also demonstrated following the administration of glucocorticoids in normal healthy subjects (Thompson et al. 1972) and in patients with hypercortisolism (Stiel et al. 1970). Similarly, in acromegaly, a high dose of dexamethasone suppressed the basal GH concentrations by more than 30% in 10 out of 15 patients (Nakagawa & Mashimo 1973). Although these results may indicate that glucocorticoids at a pharmacological concentration inhibit GH secretion in both healthy individuals and in acromegalic subjects (Nakagawa & Mashimo 1973), the site of action and its mechanism are unknown. The results of experiments performed in rats were controversial (Pecile & Müller 1966; Birge et al. 1967). The present study has clearly shown that glucocorti-
coids directly act on pituitary adenoma cells of acromegaly to suppress GH secretion.

When adenoma cells of acromegaly were co-incubated with ovine CRF and hydrocortisone, the stimulatory effect of CRF on GH secretion was partially overcome by hydrocortisone. Of interest in this regard is the observation in one experiment that the inhibitory effect of hydrocortisone was reversed by co-incubation with CRF, although CRF alone was ineffective to stimulate GH release (No. 4 in Fig. 2). These results suggest that glucocorticoids may compete with CRF to suppress GH release in pituitary adenoma cells of acromegaly. Of relevance in this context is the finding in rats that pre-treatment with dexamethasone blocks the increase in plasma ACTH levels after injection of ovine CRF (Rivier et al. 1982). In conclusion, the results of the present study suggest that CRF may stimulate GH release in some acromegalic subjects and that glucocorticoids may reverse this effect of CRF acting directly on the pituitary adenoma cells of acromegaly.

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References


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