Effect of Synthetic Ovine Corticotropin-Releasing Factor on Growth Hormone Secretion in Patients with Acromegaly

KOSHI TANAKA, TOSHIO WATABE, HYSAYOSHI YOSHIDA AND NAOKATA SHIMIZU

First Department of Medicine, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173

Abstract

In a significant proportion of patients with acromegaly, a non-specific increase in plasma growth hormone (GH) has been recognized following administration of thyrotropin-releasing hormone (TRH) or luteinizing hormone-releasing hormone (LH-RH), probably due to the lack of the specificity of the receptor in their tumor cells. In this study, the effects of corticotropin-releasing factor (CRF), a newly isolated hypothalamic hormone, in addition to TRH and LH-RH, on plasma levels of GH and the other anterior pituitary hormones were evaluated in 6 patients with acromegaly. Synthetic ovine CRF (1.0 µg/kg), TRH (500 µg) or LH-RH (100 µg) was given as an iv bolus injection, in the morning after an overnight fast. Blood specimens were taken before and after injection at intervals up to 120 min, and plasma GH, adrenocorticotropin (ACTH), thyrotropin, prolactin, luteinizing hormone, follicle-stimulating hormone and cortisol were assayed by radioimmunoassays. A non-specific rise in plasma GH was demonstrated following injection of TRH and LH-RH, in 5 of 6 and 2 of 5 patients, respectively. In all subjects, rapid rises were observed in both plasma ACTH (34.3±6.2 pg/ml at 0 min to 79.5±9.5 pg/ml at 30 min, mean±SEM) and cortisol level (9.1±1.3 µg/dl at 0 min to 23.4±1.2 µg/dl at 90 min). However, plasma levels of GH and the other anterior pituitary hormones did not change significantly after CRF injection. These results indicate that CRF specifically stimulates ACTH secretion and any non-specific response of GH to CRF appears to be an infrequent phenomenon in this disorder.

In normal subjects, thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LH-RH) specifically stimulate secretion of thyroid-stimulating hormone (TSH), and luteinizing hormone (LH) and follicle-stimulating hormone (FSH), respectively. In contrast, a non-specific increase in plasma growth hormone (GH) following the administration of TRH (Saito et al., 1971; Irie et al., 1972; Schalch et al.,...
1972; Faglia et al., 1973a; Liuzzi et al., 1974; Cantalamessa et al., 1976; Nakagawa and Obara, 1977; Hanew et al., 1980; Marek et al., 1981) or LH-RH (Faglia et al., 1973; Liuzzi et al., 1974; Cantalamessa et al., 1976; Nakagawa and Obara, 1977; Rubin et al., 1977) has been well recognized in a significant proportion of patients with acromegaly. Although the exact reasons for these non-specific responses is not fully understood as yet, some altered receptor mechanism has been postulated (Matsukura et al., 1977) in the tumor cells of the pituitary responsible for excess production of GH in this disorder.

Corticotropin-releasing factor (CRF), a hypothalamic hypophysiotropic hormone recently isolated from ovine hypothalami and subsequently synthesized (Vale et al., 1981; Spiess et al., 1981; Rivier et al., 1982), has been shown to specifically stimulate adrenocorticotropic (ACTH) secretion in normal human subjects (Grossman et al., 1982; Muller et al., 1982; Orth et al., 1983). This study was undertaken in order to assess whether CRF might likewise exert such a non-specific stimulatory effect on the secretion of GH and other anterior pituitary hormones in patients with acromegaly.

Materials and Method

Synthetic ovine CRF was purchased from The Protein Research Foundation, Osaka, Japan. The CRF preparation was dissolved in 0.05 M acetic acid containing 5 percent (w/v) mannitol in a concentration of 200 µg/ml, then sterilized with a Durapore filter (Millipore Corp., type GVWP, pore size 0.22 µm). The CRF solution was then lyophilized in 0.5 ml aliquots (100 µg) in sterile vials and stored at 4°C until used. Sterility of the lyophilized CRF was proven by the standard sterility test (Society of Japanese Pharmacopoeia, 1981). The biological activity of CRF retained in the lyophylized preparation was evaluated using a rat anterior pituitary cell monolayer culture according to the methods of Vale et al. (1972). Concentration of ACTH in the culture medium was assayed by radio-immunoassay (RIA) using reagents provided by NIAMDD. Synthetic TRH and LH-RH for injection were obtained from Daiichi Seiyaku Co., Ltd., Tokyo, Japan.

Six female patients with acromegaly were investigated after informed consent was obtained from each patient. Their age, body weight and previous treatment are shown in Table 1. 2-Bromo-α-ergocriptine (CB 154) was withdrawn 2 to 30 days prior to the present study. The experiments were performed in the morning after an overnight fast, and the patients remained inactive and supine for 30 min prior to the first blood sampling throughout each experimental period. To a vial containing 100 µg of sterilized CRF, 2.0 ml sterile normal saline was added immediately prior to injection and a dose of 1.0 µg/kg body weight was given to each patient as an intravenous bolus injection. Blood specimens were taken at -15, 0, 15, 30, 60, 90 and 120 min after injection for later determination of ACTH, GH, TSH, prolactin (PRL), LH, FSH and cortisol by RIA. 500 µg TRH or 100 µg LH-RH was similarly administered on non-consecutive days. Blood samples were obtained before and 30, 60, 90 and 120 min after injection of TRH or LH-RH and plasma concentrations of GH, TSH and PRL, and GH, LH and FSH were assayed, respectively, by RIA. Plasma ACTH and PRL were assayed using RIA kits obtained from Commissariat a l‘energie atomique, France. Plasma GH and cortisol, and TSH, LH and FSH were determined with RIA kits obtained from Eiken Immunochemical Labs., Ltd., Tokyo, Japan and Daiichi Radiisotope Labs., Ltd., Tokyo, Japan, respectively. All samples for each hormone were assayed in duplicate in the same assay. Intra-assays coefficients of variation for ACTH, GH, TSH, PRL, LH, FSH and cortisol were 6.9, 7.5, 5.5, 4.5, 7.7, 8.0 and 4.3 percent, respectively.

Results are presented as the mean±SEM unless otherwise stated. Statistical analysis was performed using Student’s t-test for paired or non-paired data where appropriate. Plasma GH responses were arbitrarily considered as positive when a rise of 50 percent or greater over basal level and a net increment greater than 10 ng/ml were observed, because considerable spontaneous fluctuation in plasma GH level has been observed in this disorder (Cryer and Daughaday, 1969; Camanni et al., 1975; Hanew et al., 1980).
Results

The concentration of bioactive CRF in the solution prepared for injection was 105.6 ± 6.2 percent of that expected, which was not significantly different from the expected concentration (p > 0.1) and indicated that no appreciable loss of bioactivity had occurred during the preparation of CRF for injection.

Responses of plasma GH, TSH and PRL to TRH are summarized in Table 1. According to the criteria described in the Methods, a significant rise in plasma GH was observed in 5 of 6 patients after TRH injection (case 1, 2, 4, 5 and 6). Changes in plasma GH, LH and FSH concentration...
after LH-RH injection are also shown in Table 1. Plasma GH levels increased significantly in 2 of 5 patients (case 3 and 5) who received LH-RH. Plasma ACTH as well as cortisol concentration rose significantly in each subject as shown in Fig. 1. The mean plasma ACTH and cortisol levels for all patients increased from the basal (0-time) levels (34.3 ± 6.2 pg/ml and 9.1 ± 1.3 μg/dl, respectively) to peak levels of 79.5 ± 9.5 pg/ml at 30 min and 23.4 ± 1.2 μg/dl at 90 min, respectively. The mean maximum increases in ACTH and cortisol (2.3 and 2.6 times the basal level, respectively) were comparable to those in normal subjects reported in recent studies (Grossman et al., 1982; Müller et al., 1982; Orth et al., 1983; Tanaka et al., 1983).

Changes in GH concentration after CRF administration are depicted in Fig. 2. Although slight increases in GH levels were observed in 2 of 6 patients (case 3 and 4), they did not appear to be significant, based on the criteria described in the Methods, since the maximum increases were only 24 and 25 percent, respectively. In another 2 patients (case 5 and 6), a slight decrease in plasma GH level was observed, but was not con-

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sidered to be significant for the same reason. Changes in the plasma concentration of TSH, PRL, LH and FSH after injection of CRF are shown in Fig. 3. There was no significant change in the concentration of any of these hormones during the experimental period. No subjective symptoms were reported by any of the patients after administration of CRF.

Discussion

In the present study, a significant rise in plasma GH was demonstrated in all of 6 patients following the administration of either TRH or LH-RH, providing evidence of an altered receptor in their pituitary tumor cells as has been postulated (Matsukura et al., 1977), although exact mechanism still remains to be clarified. The fact that plasma ACTH and cortisol increased significantly after CRF (Fig. 1), indicated that the CRF employed in this experiment was biologically active and indeed stimulated their pituitaries to secrete ACTH. However, none of the patients showed any appreciable increase in plasma GH levels after CRF injection.

There are several possible explanations for the unresponsiveness of GH to CRF in the acromegalic patients examined in this study. Firstly, any non-specific stimulatory effect of CRF on GH secretion in this disorder may be less common than that seen with TRH (Saito et al., 1971; Irie et al., 1972; Schalch et al., 1972; Faglia et al., 1973a; Liuzzi et al., 1974; Cantalamessa et al., 1976; Nakagawa and Obara, 1977; Hanew et al., 1980; Marek et al., 1981) or LH-RH (Faglia et al., 1973b; Liuzzi et al., 1974; Cantalamessa et al., 1976; Nakagawa and Obara, 1977; Rubin, et al., 1977). Thus, it is possible that an increase in GH by CRF was not demonstrated because of the rather small population evaluated in this study, and a larger number of patients may be required to establish a definite conclusion. Secondly, the dose of CRF might have been insufficient, since on a molar basis 500 μg TRH and 100 μg LH-RH are 113 and 7 times of that of the mean dose of the CRF (57.5 μg). A higher dose of CRF may provoke non-specific GH release from the acromegalic pituitary, although proper precautions seem to be indicated because several side effects including a decrease in systemic blood pressure have been reported at a doses of higher than 1.0 μg/kg (Orth et al., 1983). Thirdly, CB 154 which was given to 3 of 6 patients until 2 to 30 days prior to the experiments might have suppressed GH release that would have occurred otherwise in these patients. Oral administration of 5 mg CB 154 has been reported to suppress TRH-stimulated PRL secretion completely but not to affect TRH-stimulated GH release in acromegalic patients (Ishibashi et al., 1977). In our patients, a significant rise in plasma PRL level was observed in all 3 cases (Table 1), indicating that the CB 154 previously administered was not affecting their pituitary at the time of the experiment. Thus, the third possibility seems unlikely. Lastly, human CRF rather than ovine CRF which was used in this study, may have a different action on the tumor cells in the acromegalic pituitary and thus might induce non-specific GH secretion. Studies with human CRF, the amino acid sequence of which has been deduced recently from the nucleotide sequence of cloned DNA (Shibahara et al., 1983), may shed light on this question.

In summary, no significant rise in plasma GH was observed after the administration of synthetic ovine CRF to patients with acromegaly who exhibited considerable rise in plasma GH in response to TRH and/or LH-RH. However, the exact incidence of responsiveness of GH to synthetic ovine CRF in this disorder remained to be established.
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