NOTE

Comparative *in vivo* and *in vitro* Studies on Abnormal GH Secretion in Patients with Acromegaly

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Abstract

Eight patients with active acromegaly due to GH-producing pituitary adenoma were studied. GH secretory dynamics *in vitro* was evaluated by adding GRF, CRF, or a somatostatin analog, SMS 201-995 to the perfusate of dispersed cells from tumors. A comparison was made between the data obtained in preoperative tests for GH secretion and those obtained in experiments *in vitro*. Before operation, the GRF test (100 μ g, iv) resulted in no GH response in three of six patients examined. The CRF test (100 μ g, iv) resulted in a paradoxical GH increase in two of the same six patients. *In vitro* studies performed on adenoma cells revealed that exposure to GRF (100 ng/ml) elicited an increase in GH in seven of eight patients examined. Exposure to CRF (100 ng/ml) caused an enhanced GH secretion in four of the same eight patients. There were cases in which GH response to these hypothalamic hormones was observed *in vitro* but not *in vivo*, whereas there was only one case in which CRF caused an increase in GH *in vivo* but not *in vitro*. Thus, GH secretory dynamics was not always the same *in vivo* and *in vitro*. The discrepancy could be ascribed to the different secretory status of hypothalamic hormone (e.g., GRF or somatostatin) *in vivo* in each acromegalic patient.

In patients with acromegaly the secretory pattern of GH is often different from that of the normal. Recently, a paradoxical increase in plasma GH in response to intravenous CRF, in addition to that caused by TRH and LHRH, was reported (Pieters *et al.*, 1984a). With respect to GRF, GH response is not always the same as in the normal. Decreased response of plasma GH

or no response to GRF administered iv is frequently reported, though GH is increased either to the same as, or to a greater extent than the normal in the majority of the acromegalics (Wood *et al.*, 1983, Shibasaki *et al.*, 1984, von Werder *et al.*, 1984, Pieters *et al.*, 1984b, Gelato *et al.*, 1985, Arosio *et al.*, 1985). These abnormalities in GH secretion could be due to either a change in the hypothalamic regulatory mechanism (hypothalamic hor-

mones and/or amines) for GH secretion, the disappearance of, or aberrant appearance of, receptors for these hypothalamic hormones at the pituitary level, and/or a change in the postreceptor mechanism.

In this study secretory responses of GH to GRF and CRF were examined *in vitro* in pituitary adenomas from acromegalics, and a comparison was made with those obtained in loading tests for GH secretion before operation. It was the purpose of this study to determine to what extent GH secretion *in vitro* can reflect secretion *in vivo*, and to get further insight into the mechanism underlying abnormal GH secretion in acromegalic patients.

Materials and Methods

Patients

Eight patients (aged 37–60 yr) with active acromegaly were examined in this study. All these patients had clinical features characteristic of acromegaly, viz. elevated plasma GH and somatomedin-C, or lack of GH suppression after oral administration of 100 g glucose. The clinical and biochemical data for the eight patients are summarized in Table 1. All of these patients underwent transsphenoidal surgery and a pituitary adenoma, which was confirmed

histologically, was resected successfully in every case.

Tests on GH secretion

Before operation, tests of GH secretory dynamics were performed. These included GRF and CRF tests (100 µg, both) performed on separate occasions in six patients. The tests were started by administering these agents iv as a bolus at 800 h after an overnight fast and rest for 60 min. Human GRF and human CRF were purchased from the Protein Institute (Osaka, Japan). Before the tests, an indwelling needle for administration of test substances and blood collection was inserted into a forearm vein and kept open by a slow 0.9% saline infusion. Plasma was separated and stored at -30°C until assay. When plasma GH was increased by 50% above the basal level at least in two samples within 60 min after injection, it was judged as an increase (positive response).

Cell culture

Pituitary adenoma tissues obtained at transsphenoidal surgery were minced and then enzymatically dispersed with collagenase, Dispase and DNase according to Ceda *et al.* (Ceda *et al.*, 1985). Dispersed adenoma cells were then cultured for four to five days until used in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal calf serum at 37°C in an atmosphere of 95% air and 5% CO₂.

Table 1. Clinical and biochemical data for 8 patients with active acromegaly

Patient no	Sex	Age (yr)	Plasma GH (ng/ml)	Somatomedin-C (U/ml)	Histology of adenoma
1	F	59	64.4	—	acidophilic
2	M	54	32.0	3.11	acidophilic
3	M	55	31.7	—	acidophilic
4	M	46	34.5	1.67	acidophilic
5	M	37	36.7	4.04	acidophilic
6	F	60	33.2	—	acidophilic
7	M	59	40.1	3.94	acidophilic
8	M	35	17.7	3.08	acidophilic
Mean		50.6	36.3	3.17	
±SE		3.6	4.6	0.42	

The mean of several determinations is shown.

In patient 1, plasma PRL is also increased (67.8 ng/ml).

—; not determined.

Table 2. GH responses to GRF *in vivo* and *in vitro*

Patient	<i>In vivo</i>		Response	<i>In vitro</i>		Response
	Basal GH (ng/ml)	Peak GH (ng/ml)		Basal GH (ng/fract)	Peak GH (ng/fract)	
1	74.3	415.1 (558)	+	8.3	18.6 (224)	+
2	34.4	193.4 (562)	+	7.1	16.2 (228)	+
3	—	—	—	13.6	25.7 (188)	+
4	19.2	26.8 (139)	—	7.2	16.1 (223)	+
5	39.2	48.0 (124)	—	24.4	38.3 (156)	+
6	—	—	—	17.5	18.7 (106)	—
7	38.7	42.0 (108)	—	11.7	21.5 (182)	+
8	13.4	24.5 (182)	+	6.6	11.5 (174)	+

In patients 3 and 6, GRF test was not performed preoperatively. A GH increase to more than 50% over the basal was judged as a positive response, +, and that less than 50% as negative, —. () ; % of basal GH.

Perfusion experiments

Perfusion experiments were performed on eight adenomas. A perfusion column was prepared with a syringe and the volume of the perfusion chamber was adjusted to 1.0 ml. As a perfusion medium, Krebs-Ringer bicarbonate buffer containing 0.2% glucose and 0.1% bovine serum albumin was used. The medium was bubbled with 95% O₂ and 5% CO₂ throughout the experiments. About 4×10^6 cells were gently mixed with Sephadex G-10 previously equilibrated with the medium in the column according to the method described by Gillies and Lowry (Gillies and Lowry., 1978) with a modification. The flow rate was 0.5 ml/min. The system was allowed to equilibrate for 2 h before the start of effluent collection, and 1.5 ml fractions were collected at 3 min intervals. *In vitro* GH secretory dynamics was evaluated by adding to the perfusate CRF (100 ng/ml), GRF (1 or 100 ng/ml), and a somatostatin analog, SMS 201-995 (100 ng/ml, Sandoz, Switzerland) alone or in combination as a pulse of 4 ml (8 min) in random order. In addition to these agents, VIP, LHRH and TRH were also added, though these data are not shown for simplification. Intervals between pulses were approximately 60 min. At the end of each experiment the cells were exposed to a pulse of concentrated K (50 mM) in order to determine the viability of the perfused cells. The effluents were stored at -30°C until assay for GH. The mean of GH concentrations in several fractions immediately before a pulse was regarded as the basal level.

When the GH concentration exceeded 150% of the basal level at an appropriate time after a pulse, it was judged as an increase (positive response).

Hormone assays

Plasma GH and the amount of GH in the effluent were determined by radioimmunoassay as reported previously (Maeda *et al.*, 1976). GH was kindly provided by Dr. A. Parlow (National Pituitary Agency) and Dr. H. G. Friesen (University of Manitoba, Canada).

All the samples from a given experiment were run in the same assay. Intra- and inter-assay coefficients of variation were both less than 10%.

Results

In vivo GH secretory dynamics

The GRF test was performed in six acromegalic patients. GRF elicited a positive response (an increase) in plasma GH in three, but a negative response in plasma GH in the other three of six patients (Table 2). Plasma GH was increased from a basal level of 73.3 ng/ml to 415.1 ng/ml, from 34.4 ng/ml to 193.4 ng/ml and from 13.4 ng/ml to 24.5 ng/ml in patients 1, 2 and 8, respectively. The CRF test was also performed in the same six patients.

Table 3. GH responses to CRF *in vivo* and *in vitro*

Patient	<i>In vivo</i>		Response	<i>In vitro</i>		Response
	Basal GH (ng/ml)	Peak GH (ng/ml)		Basal GH (ng/fract)	Peak GH (ng/fract)	
1	46.9	84.8 (180)	+	8.9	9.2 (103)	—
2	18.5	48.5 (262)	+	7.6	18.2 (239)	+
3	—	—	—	14.4	16.7 (115)	—
4	19.7	19.9 (101)	—	8.1	19.8 (244)	+
5	36.3	40.3 (110)	—	26.2	53.4 (203)	+
6	—	—	—	17.5	19.3 (110)	—
7	38.9	41.5 (106)	—	10.3	19.4 (188)	+
8	16.4	21.1 (128)	—	7.4	6.5 (101)	—

In patients 3 and 6, the CRF test was not performed preoperatively. A GH increase to more than 50% over the basal was judged as a positive response, +, and that less than 50% as negative, —. () ; % of basal GH.

Fig. 1. Effect of GRF, SMS 201-995 and 50 mM K⁺ on GH secretion from somatotroph adenoma *in vitro*. Results for patient 5 are shown.

CRF administration resulted in a GH increase in two patients (Table 3). In contrast to responses to GRF, CRF elicited a smaller GH increase. Plasma GH levels changed from 46.9 ng/ml to 84.8 ng/ml and from 18.5 ng/ml to 48.5 ng/ml in patients 1 and 2, respectively, both of whom also responded to GRF.

In vitro GH secretory dynamics

In a preliminary experiment, after an equilibration period of 2 h, basal GH levels were stable without marked spontaneous

fluctuations throughout the experiment. At the end of the experiments, the addition of 50 mM K⁺ elicited a burst increase in GH in every case, indicating that the cells were viable for the period (Fig. 1).

The effect of GRF on GH release *in vitro* was examined in all of the eight cases. The GH concentration was increased in response to GRF (100 ng/ml) in seven cases (Table 2). The increases were to approximately twice the basal level and peaked approximately 5 min after the beginning of the pulse. GH returned to the

basal level approximately 15 min after the end of the pulse. GRF at a lower dose (1 ng/ml) also elicited an increase in GH in the perifusate in these seven cases. A representative change in the GH concentration in response to 100 ng/ml GRF is shown in Fig. 1.

Fig. 2. Effect of CRF on GH secretion from a somatotroph adenoma *in vitro*. Results for patient 2 are shown.

The effect of CRF was examined in eight cases *in vitro*. A GH increase was elicited in four of eight cases in response to CRF (100 ng/ml) (Table 3). The magnitude and time course of the GH increase were comparable with those of GRF (Fig. 2). Whenever an increase in GH was elicited by CRF, a GH response to GRF was also observed *in vitro*.

In some cases an increase in GH was elicited *in vitro* but not *in vivo* by GRF (Patients 4, 5 and 7, Table 2). It was probable that a GH increase to be caused by GRF was inhibited by endogenous somatostatin *in vivo* in these cases. Therefore, SMS 201-995 (100 ng/ml) was added simultaneously with GRF (100 ng/ml) to the

perifusate in cases 4, 5 and 7; to evaluate the sensitivity of these adenomas to somatostatin. In these three cases, an increase in GH, which was caused by GRF alone, was abolished completely by simultaneous administration of SMS 201-995. Further, GH was increased over the basal level 15 min after the termination of simultaneous exposure to SMS 201-995 and GRF (Fig. 1).

On the other hand, an increase in GH was induced *in vivo* but not *in vitro* by CRF in patient 1 (Table 3). It is possible that endogenous GRF sensitized the adenoma to exogenous *in vivo* CRF resulting in a GH increase. Therefore, the effect of CRF (100 ng/ml) in the presence of GRF administered continuously at a low dose (1 ng/ml) was examined in case 1. GRF (1 ng/ml) caused a GH increase, which peaked 15 min after the beginning of the pulse and declined thereafter despite its continued presence. However, CRF added during the

Fig. 3. Effect of CRF on GH secretion from a somatotroph adenoma in the presence of GRF in a lower dose *in vitro*. Results for Patient 1 are shown.

latter half of GRF exposure did not effect a further decline in the GH concentration (Fig. 3).

In some cases (Patients 4, 5 and 7) an increase in GH due to CRF was elicited *in vitro* but not *in vivo* (Table 3). It was probable that endogenous somatostatin inhibited the GH response to CRF *in vivo*. As shown above, SMS 201-995 inhibited a GRF-induced GH increase when added to the perfusate simultaneously with GRF. However, the effect of SMS 201-995 on GH secretion induced by CRF was not examined.

Discussion

In this study a bolus iv injection of GRF, unexpectedly, did not cause an increase in GH in half of the patients (three of six patients). An increase in GH either to the same or a greater extent as in the normal has been reported in the majority of acromegalics. In our patients the frequency of decreased or no response to GRF was higher than those reported previously (Wood *et al.*, 1983, Shibasaki *et al.*, 1984, von Werder *et al.*, 1984, Pieters *et al.*, 1984b, Gelato *et al.*, 1985, Arosio *et al.*, 1985).

The reduced or no GH response to GRF in acromegalics could be due to a change in the hypothalamic regulatory mechanism, disappearance of GRF receptor in the pituitary, or changes in the postreceptor mechanism. In addition, it has been reported that GH response to GRF is usually suppressed in patients with an ectopic GRF-producing tumor, a rare form of acromegaly (Schulte *et al.*, 1985). Although increased GH in this study was due to a pituitary adenoma in each case, excessive hypothalamic GRF might have been responsible for no or decreased GH response to exogenous GRF.

In this study GRF elicited a GH increase *in vitro* in seven of eight patients.

Thus, the *in vivo* frequency of negative GH response was much higher than that observed *in vitro*. This type of discrepancy might be explained by a factor(s) which interfered with GH response to GRF *in vivo*. First, endogenous GRF in excess might have suppressed GH response to exogenous GRF *in vivo* as mentioned above. However, a definite conclusion cannot be formed until the GRF in the portal system can be measured accurately. Second, endogenous somatostatin in excess might be responsible. Endogenous somatostatin might be increased as a result of feedback in a group of acromegalic patients. In this group, an excess of endogenous somatostatin would interfere with GH response to GRF, as long as an adenoma was sensitive to somatostatin. To evaluate this possibility, an analog of somatostatin, SMS 201-995, which was demonstrated to bind specifically to somatostatin receptors in human pituitary (Reubi and Landolt, 1984), was used *in vitro* in this study. An increase in GH caused by GRF (100 ng/ml), was abolished completely simultaneous administration of SMS 201-995 (100 ng/ml) with GRF, indicating that these adenomas were sensitive to somatostatin. However, no definite conclusion on the possible involvement of endogenous somatostatin can be drawn until the somatostatin concentration in the portal blood is measured.

In this study CRF administered iv as a bolus elicited an increase in GH in two of six acromegalics. An increase in plasma GH in response to CRF has been also reported in acromegalics (Pieters *et al.*, 1984a) and in patients with primary affective disorders (Gold *et al.*, 1983). The frequency of a paradoxical increase in GH in response to CRF in this study was comparable to that in acromegalic reported previously (Pieters *et al.*, 1984a), but much lower than that caused by TRH (Faglia *et al.*, 1973a).

The paradoxical increase caused by CRF

might also be ascribed to a change in the hypothalamic regulatory mechanism (e.g., contribution of GRF exposure to GH increase by TRH, Sartorio *et al.*, 1986), the aberrant appearance of receptor for CRF in somatotrophs (Pieters *et al.*, 1984a) as postulated for the mechanism of paradoxical responses by TRH and LHRH (Faglia *et al.*, 1973a, Faglia *et al.*, 1973b, Matsukura *et al.*, 1977) or changes in the postreceptor mechanism. The finding reported by Pieters *et al.*, (1984a) might indicate that basal GH levels determine responsiveness to CRF. In this study, basal GH was high in one and low in another responsive patient. Taken together, these data suggest that the basal GH level is not a major determinant of GH responsiveness to CRF.

In response to CRF, an increase in GH was elicited both *in vivo* and *in vitro* in one patient proving the site of CRF action on the pituitary (Patient 2). In another patient an increase in GH was induced *in vivo* but not *in vitro* by CRF (patient 1). It was possible that endogenous GRF sensitized the adenoma to exogenous CRF in this patient as postulated for paradoxical GH response to TRH (Thorner *et al.*, 1982, Schulte *et al.*, 1985). Therefore, the effect of CRF (100 ng/ml) was examined in the presence of GRF in a low dose (1 ng/ml) administered continuously. CRF, added during the latter half of GRF exposure, did not induce a GH response, though GRF (1 ng/ml) alone caused a GH increase. The size of the tumor did not allow us to further examine various conditions such as the use of different doses of GRF and CRF, and timing of CRF addition. Therefore, it might be premature to draw a conclusion on the possible involvement of endogenous GRF in this patient.

The frequency of positive GH response to CRF *in vitro* was also higher than that observed *in vivo*. CRF elicited a GH increase in four of eight patients *in vitro*, but

in only two of six patients *in vivo*. This discrepancy might be due to the fact that endogenous somatostatin interfered with a GH increase in response to CRF *in vivo* as mentioned above in connection with GH response to GRF. However, the effect of somatostatin or SMS 201-995 on the CRF-induced GH increase was not examined in these cases because of the limited size of tumor amount available.

The discrepancy between GH responses *in vivo* and *in vitro* to GRF or CRF in this study suggests that the involvement of endogenous somatostatin and/or GRF in the regulation of GH secretion in acromegalics might be qualitatively different from patient to patient. The so-called paradoxical GH response in patients with acromegaly might reflect the secretory status of these hypothalamic hormones *in vivo*. In order to further verify this hypothesis it is necessary to evaluate the level of these hypothalamic hormones in the portal blood directly or indirectly.

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