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Persistence of somatostatinergic tone in acromegaly

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It is a matter of debate whether hypothalamic somatostatin (SRIH) secretion in acromegaly is preserved and still regulated by the physiological feedback mechanisms of growth hormone (GH) and insulin-like growth factor I. To gather further information on this, the reproducibility of plasma GH changes induced by growth hormone-releasing hormone (GHRH) administration was evaluated in 15 acromegalic patients. There was a highly significant correlation between the peak/basal ratio (P/B) GH response in the 15 patients administered GHRH on two separate occasions ($r = 0.99$, $p < 0.001$). The test was performed also before and after the administration of drugs able to inhibit or stimulate hypothalamic SRIH release, by activating (pyridostigmine) or inhibiting (pirenzepine) cholinergic pathways, respectively. The GHRH-induced GH response (P/B = 2, range 1.1–26.1) was increased significantly by pyridostigmine pretreatment in 30 patients (P/B = 2.6, range 1.3–34.8; $p = 0.0045$). In nine out of 30 patients an increase of greater than 2 SD of within-subject GHRH variability was observed in response to GHRH plus pyridostigmine when compared to GHRH alone. An inverse correlation ($r = -0.37$, $p < 0.05$) was observed between GH response to GHRH alone and after pyridostigmine pretreatment. On the contrary, no change of GHRH-induced GH response was observed in 12 patients after pirenzepine pretreatment (P/B = 1.9, range 1.1–5 and P/B = 2, range 1.3–6 without and after pirenzepine pretreatment, respectively). These data suggest that in acromegaly the somatostatinergic tone does not seem to fluctuate, and that it can be inhibited often by cholinergic pathway activation but not increased further by cholinergic suppression.

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The GHRH-induced GH response has been shown to be variable among acromegalic patients (1,2), but we are not aware of any studies on the intrasubject reproducibility of this response. As the wide intrasubject variability observed in GHRH-induced GH levels in normal subjects has been attributed to the fluctuations of hypothalamic somatostatin (SRIH) tone (3), gathering data on this matter could, in fact, indirectly shed some light on the somatostatinergic tone in acromegaly, which is still a matter of debate.

A reduction of hypothalamic SRIH secretion was suggested as being the primary cause of acromegaly (4). Recently, attention has focused on primary pituitary pathogenesis of this disease, the hypothesis being that an excess of hypothalamic SRIH occurs provided that the short-loop feedback between GH and SRIH secretion (5) is well functioning. There is, indeed, evidence that hypothalamic control of GH secretion may be preserved in acromegalic patients, because their GH levels often increase after stimuli such as arginine, insulin and exercise, which modulate GHRH and/or SRIH release at the hypothalamic level (6).

To obtain information on somatostatinergic tone in acromegaly, we studied the reproducibility of the GH response to GHRH in acromegalic patients. Somatostatinergic tone also was evaluated indirectly in a larger group of patients after pharmacological manipulations: GHRH-induced GH secretion was studied after the administration of pyridostigmine (Pyr), putatively inhibiting SRIH secretion by activation of cholinergic pathways (7), and pirenzepine (Pz), an antimuscarinic drug that is supposed to stimulate SRIH secretion (8).

Materials and methods

Patients

Thirty patients (11 males and 19 females, aged 22–76 years, median 51) with active acromegaly (according to increased GH, not suppressible by glucose load, and high IGF-I levels) entered the study. Individual demographic and clinical data are reported in Table 1. No patient was hyperprolactinemic. Any drug treatment aimed at lowering GH hypersecretion or potentially capable of interfering with GH secretion was withdrawn at least 4 weeks before the start of the study, as part of

*Deceased.

Table 1. Demographic and clinical data.

Case no.	Sex	Age (years)	Radiology[a]	Previous treatment			IGF-I (μg/l)
				Neurosurgery[b]	Radiotherapy (year)	Replacement[c] therapy	
1	M	69	M−	−	−	−	600
2	F	69	E−	−	−	−	495
3	F	62	E−	−	1982	−	760
4	M	65	N−	−	1991	A	520
5	F	39	M+	TNS	−	−	756
6	F	56	M−	−	−	−	607
7	F	76	M+	−	1976	−	675
8	F	54	M+	TNS	1990	AT	510
9	F	44	M+	TNS, TC	1984	ATG	807
10	F	44	M+	TC	1990	AT	616
11	M	36	μ−	TNS	1986	−	540
12	F	37	M+	−	−	−	540
13	M	22	M+	TNS	1990	AG	843
14	F	56	M−	TNS	1990	−	602
15	M	65	M+	−	1976	−	700
16	F	60	E−	−	−	−	652
17	M	59	M+	TNS	1984	AG	525
18	F	70	E−	−	−	−	945
19	F	68	M+	−	−	−	540
20	F	31	M+	TNS	−	A	562
21	F	68	M−	−	−	−	495
22	M	46	M+	TNS	1990	G	1494
23	F	66	μ−	−	−	−	816
24	F	51	M+	−	−	−	610
25	M	50	M−	TNS	−	−	690
26	F	52	μ−	−	−	−	610
27	M	35	M−	−	−	A	540
28	M	40	M+	−	−	−	1071
29	M	45	M+	−	−	−	610
30	F	43	μ−	−	−	−	715

[a] N: normal; M: macroadenoma; μ: microadenoma; E: empty sella; '+' and '−' indicate the presence or not of suprasellar extension, respectively.
[b] TNS: trans-sphenoidal; TC: trans-cranial.
[c] A: hydrocortisone; T: L-thyroxine; G: gonadal steroids.

periodic off-treatment evaluation of disease. Each patient gave informed consent after full explanation of the purpose of the study and the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Protocol

Fifteen patients (cases 1–13, 15, 22) underwent the GHRH (1–44) test (UCB, Bruxelles; 100 μg iv at time 0) twice on different days (at least 2 days apart, range 2 days–21 months). No patient was submitted to major procedures (neurosurgery or radiotherapy) between the two tests.

All 30 patients randomly underwent tests with GHRH alone and after pretreatment with Pyr (120 mg administered po 60 min before GHRH injection), each test being separated by at least 7 days. In 12 of these patients (cases 1–12) the GHRH test was repeated after pretreatment with Pz (50 mg administered po 120 min before GHRH injection). In our preliminary study these doses of Pyr and Pz were effective in increasing or lowering, respectively, plasma GH response to GHRH in normal subjects, without major side effects. At 08.00 h after an overnight fast and rest, while the patients were supine and awake, an indwelling needle was inserted in an antecubital vein and kept patent by slow saline infusion. After two basal samples (−30 min, 0 min), GHRH was injected and blood samples were collected after 15, 30, 45, 60, 90 and 120 min. When Pyr or Pz were given, two basal plasma samples were collected at −30 and 0 min before drug administration and thereafter every 30 min until GHRH injection. Blood samples collected at 15-min intervals over a period of 120 min during saline infusion on another day were used as control.

In each blood sample, GH, PRL and TSH values were assayed.

All patients had previously undergone acute tests with TRH (200 μg iv), bromocriptine (2.5 mg po) and octreotide (100 μg sc), with methods described previously (3).

Methods

Growth hormone, PRL, TSH and IGF-I were assayed in duplicate by immunoenzymatic assay, kinetic enzyme immunometric assay, solid phase two-site fluoroimmunometric assay and RIA after acid–ethanol extraction, respectively. Plasma hormone concentrations were determined in each patient in a single run. Reagents were provided by Sorin (Saluggia, Italy) for GH, DPC (Los Angeles, CA, USA) for PRL, Wallac-Oy (Turku, Finland) for TSH and Nichols Institute (San Juan Capistrano, CA, USA) for IGF-I. Standards were calibrated against first IS 80/505 (1 ng = 2 μIU) for GH, WHO first IRP 75/504 for PRL, WHO second IRP 80/558 for TSH and WHO IRP 87/518 for IGF-I. Normal values for adult patients in our laboratory are less than 5 μg/l for GH, less than 18 μg/l for PRL, 0.1–3.5 mU/l for TSH and 123–463 μg/l for IGF-I. Within-assay coefficients of variation for GH were 4.1% and 4.8% at GH concentrations near 10 and 25 μg/l, respectively.

Statistical analysis

Values were expressed as medians and ranges. The GH responses to GHRH, GHRH plus Pyr and GHRH plus Pz were evaluated as the ratio between peak and baseline (P/B).

Analysis of the results was performed by the following tests: reproducibility of the GHRH test was evaluated by the Pearson correlation, comparison of P/B ratios by the Wilcoxon rank test, comparison of results obtained among various subgroups of patients by the Mann–Whitney or Fisher test (for numerical and dichotomic results, respectively) and correlations by the Spearman test, as appropriate. Owing to wide variations, the comparison between P/B ratios after each test was repeated also after log transformation of raw data; $p < 0.05$ was considered to be statistically significant.

Results

No significant differences among basal GH levels were observed before GHRH administrations or during saline infusion (data not shown).

Growth hormone-releasing hormone elicited a significant increase in GH levels as compared to baseline values (basal 21.8 μg/l, range 6–220; peak 48 μg/l, range 8.3–634; $p < 0.00001$). An analytical evaluation of the individual responses to GHRH showed a wide intersubject variability, ranging from 1.1 to 26.1 (expressed as P/B).

On the contrary, a highly significant correlation was observed between P/B GH response in the 15 patients administered GHRH on two separate occasions ($r = 0.99$, $p < 0.001$) (Fig. 1). Basal GH levels were no different between the two tests. These data were utilized for evaluation of pharmacological manipulation of cholinergic pathways. After transforming the results of the second GHRH test into a ratio of the first, the mean and standard deviation of this ratio were calculated; the SD was 0.22 for the ratio of P/B GH levels.

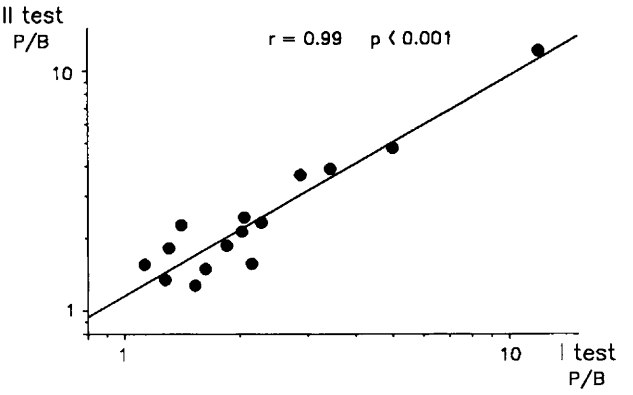

Fig. 1. Reproducibility of GHRH test, expressed as peak/basal (P/B) ratio, in 15 patients.

Pretreatment with Pyr did not change basal GH levels but significantly increased the GH response to GHRH (P/B = 2, range 1.1–26.1 for GHRH alone and P/B = 2.6, range 1.3–34.8 for GHRH plus Pyr; $p = 0.0045$). In 9 out of 30 patients (cases 1, 4, 7–10, 16, 19, 20), analytical evaluation of these data showed a potentiation of GH response to GHRH after the Pyr pretreatment greater than 2 SD (i.e. greater than 0.44) when compared to the response to GHRH alone, whereas the opposite phenomenon was observed in only one patient (case 21) (Fig. 2). No significant difference was observed in responsiveness to Pyr pretreatment among

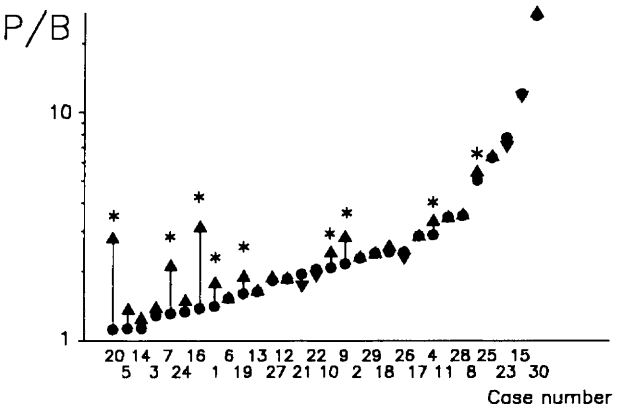

Fig. 2. Individual data of GH expressed as peak/basal (P/B) ratio in 30 patients (log scale). Each line is for a single patient, whose case number is reported in the lower part of the figure. Data are ranked in increasing order according to GH response to GHRH alone (filled circles). Arrows show variations in GHRH-induced P/B ratios after pyridostigmine pretreatment. Asterisks indicate patients in whom this variation had increased greater than 2 SD when compared to GHRH administration alone.

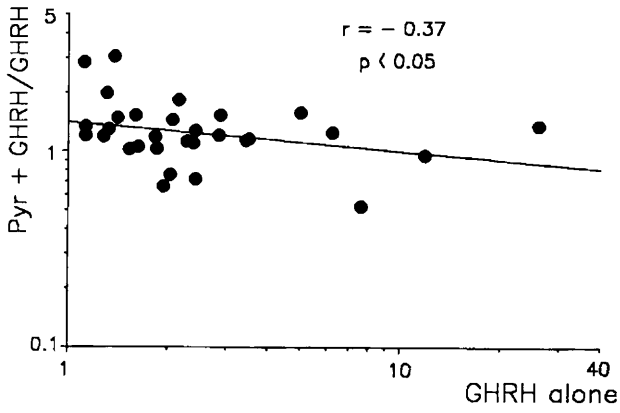

Fig. 3. Inverse correlation between GH response to GHRH alone (expressed as peak/basal (P/B) ratio on horizontal axis) and potentiation of GHRH-induced GH response after pyridostigmine (Pyr) pretreatment (expressed as ratio between P/B after GHRH plus Pyr and after GHRH alone on vertical axis).

patients treated previously or not by neurosurgery and/or radiotherapy, or among patients whose GH levels were responsive or not to acute TRH, bromocriptine or octreotide administration. No significant difference was observed in GH response to GHRH after Pyr pretreatment if patients were divided according to sex.

A statistically significant inverse correlation ($r = -0.37$, $p < 0.05$) was observed between GHRH-induced GH response (evaluated as P/B) and the potentiation of this response by Pyr pretreatment (evaluated as ratio between P/B after Pyr plus GHRH and P/B after GHRH alone) (Fig. 3).

No correlation was found between age and GH response to GHRH or Pyr potentiation of GHRH-induced GH response.

No significant change was observed in GHRH-induced GH response after pretreatment with Pz in 12 patients (P/B = 1.9, range 1.1–5 and P/B = 2, range 1.3–6 without and after Pz pretreatment, respectively). The analytical evaluation of these data showed that in no patient did Pz pretreatment cause inhibition of GH response to GHRH, whereas in two patients (cases 1 and 7) an increase was observed.

No significant variation was observed in PRL and TSH levels after each test (data not shown).

No major side effect was observed in any patient during the tests.

Discussion

A wide intersubject variability in the GH-releasing effect of GHRH was observed among our patients, as in previous studies (2, 3). It is to be noted that this response is quite reproducible in the single acromegalic subjects of this series, as shown by the highly significant correlation between the P/B GH response in patients administered GHRH on two separate occasions. This is in contrast with normal subjects, in whom a wide intrasubject variability of GHRH-induced GH response also has been shown and attributed to variations of endogenous SRIH secretion (1). Thus, the first conclusion that could be drawn from our data is that SRIH levels do not seem to fluctuate in acromegalic subjects. However, both high and low levels of SRIH might account for the reproducibility of the GHRH test in acromegaly. The results obtained by pharmacological manipulation of cholinergic pathways allowed us to gain further insight into this topic.

In normal subjects, Pyr increases the GH response to GHRH (9). On the basis of indirect animal studies it is widely accepted that the stimulation of GH secretion by the cholinergic system is mediated by an inhibition of hypothalamic somatostatin release (7), even though Magnan et al. (10) showed direct GHRH stimulation by the cholinergic agonist neostigmine in sheep. Our data are in contrast to Arvat et al. (11), who did not observe any effect of Pyr pretreatment on GHRH-induced GH release in acromegalic patients. If an arbitrary cut-off of 2 SD of within-subject GHRH variability is considered, Pyr potentiation of GHRH-induced GH response occurred in nine out of 30 patients from this series. A certain degree of hypothalamic SRIH secretion under inhibitory cholinergic control thus may persist in acromegaly. The wide intersubject variability of GHRH-induced GH response in acromegaly might, in consequence, be related to the degree of hypothalamic SRIH secretion. Alternatively, we may hypothesize in this group of patients about the occurrence of Gsp oncogene mutation that has been reported in nearly 35% of GH-secreting adenomas, with a poor response to GHRH coupled with a good response to SRIH (12). In our patients the significant inverse correlation observed between the magnitude of the Pyr potentiation of GHRH-induced GH response and the GH responsiveness to GHRH alone (i.e. the lowest the GH response to GHRH, the greatest the magnitude of Pyr potentiation) is in agreement with the former suggestion.

No difference between sexes was observed in sensitivity of the GHRH-induced GH response to Pyr potentiation, in contrast with the data reported in normal subjects by Barbarino and colleagues (13). It is worthwhile noting, however, that most females in our series were postmenopausal.

What about the non-responders, which were the majority in our series? They do not appear to be a separate population because there was a continuum from the absolute non-responders up to patients whose potentiation is of great magnitude. As neither tumor size nor duration of disease could account for the different pattern, we can hypothesize in the non-responder patients a derangement of cholinergic pathways or the predominance of alterations at the cellular level, such as the density of receptors.

The antagonism exerted by Pz on cholinergic neurotransmission did not inhibit significantly the

GH-releasing effect of GHRH. These findings are in agreement with a previous study of acromegalic patients by Pietschmann et al. (14), which showed the ineffectiveness of atropine in changing the GH response after GHRH. On the other hand, our results are in partial conflict with the reduction of GHRH-induced GH release observed in two out of five acromegalic patients by Massara et al. (9) after Pz pretreatment.

In contrast with data observed in normal subjects (15), the ineffectiveness of Pz in reducing GH response to GHRH in our study could be due to the persistence in acromegaly of the physiological reciprocal regulation between GH and IGF-I levels and SRIH secretion. It was, indeed, shown in rats that hypothalamic SRIH secretion increases after IGF-I and GH administration (16). Thus, we may hypothesize that in acromegaly SRIH secretion, when preserved, is already maximally activated by high endogenous GH and IGF-I levels and therefore it cannot be stimulated further by Pz-induced inhibition of cholinergic tone.

In conclusion, although a derangement of the cholinergic pathways or of the coupling between cholinergic and somatostatinergic pathways cannot be ruled out, our study suggests that in acromegaly SRIH secretion does not fluctuate because it is impaired or maximally stimulated. In the latter case, cholinergic neurotransmission can still inhibit, but not further stimulate, hypothalamic SRIH.

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