

Growth Hormone Release Inhibiting Hormone in Acromegaly

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British Medical Journal, 1974, 1, 352-355

Summary

Growth hormone release inhibiting hormone (GHRH) was administered by constant infusion over 75 minutes to eight acromegalic patients at different doses. 100 to 1,000 μg were equally effective in reducing circulating growth hormone (GH) levels; 25 μg lowered GH levels in only five patients, and at this dose the extent of the fall was smaller than from doses of 100 μg or more. 10 μg was ineffective. Injection of single doses of 500 μg by intravenous, subcutaneous, and intramuscular routes caused only small and transient reductions in GH levels, though the effect was improved by injecting the hormone intramuscularly in 2 ml of 16% gelatin. Injection of a suspension of 4 mg GHRH in 1 ml of arachis oil lowered growth hormone levels for between three and four hours.

In four acromegalic patients an oral 50-g glucose tolerance test was performed during a continuous infusion of either saline or 1,000 μg GHRH. The "paradoxical" rise in growth hormone seen in these patients during the saline infusion was suppressed by GHRH. The blood glucose responses were, moreover, modified by GHRH in that the peak was delayed and occurred at the end of the infusion in each case. A "normal" glucose tolerance curve was converted to a "diabetic" type of response in two patients. This effect could be accounted for by the inhibition of insulin secretion known to occur with large doses of GHRH.

We speculate that acromegaly may be primarily a hypothalamic disease due to deficiency of GHRH resulting in excessive secretion of growth hormone from the pituitary and adenoma formation due to inappropriate and prolonged stimulation of the pituitary.

Introduction

We previously reported the effects of the cyclic form of the synthetic tetradecapeptide growth hormone release inhibiting hormone (GHRH), described by Brazeau *et al.* (1973), in normal subjects and in patients with acromegaly (Hall *et al.*, 1973). An infusion of 500 μg of that material over 75 minutes was found to inhibit pituitary growth hormone (GH) secre-

tion in response to insulin-induced hypoglycaemia in normal men and was also effective in lowering circulating GH levels in the three acromegalic patients tested. Siler *et al.* (1973) also showed that GHRH will inhibit GH secretion in normal subjects after the administration of arginine or levodopa.

The aim of this study was to establish the dose-response relationship between the inhibiting hormone and the reduction of GH levels in acromegaly with a view to defining the optimal dose for therapy. Preliminary observations were also made on the effects of GHRH on blood sugar levels in view of the possible therapeutic value in those diseases associated with raised circulating levels of GH. Single-dose administration of GHRH by differing routes and attempts to prolong the action of single doses are also reported.

Patients and Methods

Synthetic GHRH prepared in the cyclic form and purified as described by Coy *et al.* (1973) was used in these studies.

Dose-response Study.—Eight acromegalic patients (six men and two women) aged 25 to 59 years volunteered to participate in the study, written consent being obtained in each case. All had radiological evidence of an enlarged pituitary fossa; five had received external irradiation to the pituitary, one had received external irradiation and an yttrium implant, and the other two were untreated. Each patient received 1,000, 500, 250, and 100 μg GHRH on different occasions infused over 75 minutes in a single-blind, random cross-over design, so that each patient acted as his own control. After the initial studies it became apparent that 100 μg GHRH was as effective as 1,000 μg in suppressing the release of GH. The same subjects were therefore given 25- μg and 10- μg infusions over the same length of time three to four weeks after the first series of tests. In the first and second series of observations the experiments were carried out at intervals of 24 to 48 hours. Samples for plasma GH assay were withdrawn from an indwelling venous cannula (inserted 30 minutes earlier) 15 minutes before and then at intervals for 150 minutes after the start of the infusion. GHRH was given in 50 ml of 0.9% NaCl by means of a constant infusion pump. Plasma was separated immediately and stored at -20°C until assayed. Samples taken from each patient during the infusions of 100 to 1,000 μg and 10 and 25 μg were measured in the same assay.

Effects of Differing Routes of Administration and Delayed-release Preparations.—A 59-year-old woman with acromegaly (case 3) had an indwelling venous cannula inserted 45 minutes before receiving single injections of 500 μg GHRH. These were given on separate occasions intravenously, subcutaneously, and intramuscularly. Blood was sampled 15 minutes before each injection, at zero time, and at intervals for 240 minutes thereafter. The intramuscular injection was later repeated with 500 μg GHRH suspended in 2 ml of warm 16% gelatin. Tests were performed on successive days and all samples were assayed in the same batch. A man aged 38 with acromegaly (case 5) received an intramuscular injection of 4 mg GHRH suspended in arachis oil. Blood samples were withdrawn at intervals over the next 30 hours.

Effect of Infusion of GHRH on GH and Blood Glucose Response during Oral Glucose Tolerance Tests.—Four acromegalic patients (three women and one man aged 49-59 years)

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without clinical or chemical diabetes mellitus but who were known to show "paradoxical" GH rises in response to oral glucose loading were studied on two occasions after the ingestion of glucose. All patients were recumbent and at rest for 30 minutes before and during the study. Blood samples were withdrawn via an indwelling intravenous cannula before the administration of 50 g glucose and at 30-minute intervals thereafter for 150 minutes. During this time each patient received an infusion of either 50 ml 0.9% NaCl or 1,000 µg GHRH in the same volume of saline. The specimens from each patient were assayed in the same batch.

Assays.—Plasma GH was measured by a double antibody radioimmunoassay and the results were expressed in ng of M.R.C. standard 69/46 per ml. This standard was also used for iodination. The maximum coefficient of variation between assays was 15%. Blood glucose was measured by a neocuprine method (Technicon).

Results

Dose-response Study.—When the effects of differing doses of GHRH on circulating GH levels were compared it was seen that there was no significant difference between 1,000, 500, 250, and 100 µg given over 75 minutes (fig. 1). The individual responses to 500 µg are shown in fig. 2. The GH rebound phenomenon after the end of the injection, reported previously, was again shown. When the dose was reduced to 25 µg, however, plasma GH levels fell in only five of the eight patients, but the extent of the fall was not as great as when 100 µg was used. In the five responsive patients GH levels fell from basal levels of 60, 60, 14, 22, and 23 ng/ml to 34, 21, 8, 19, and 14 ng/ml respectively at the end of the infusions. None of the eight subjects showed a reduction in circulating GH levels in response to 10 µg GHRH.

Effects of Differing Routes of Administration and Delayed-release Preparations.—The duration of action of the single

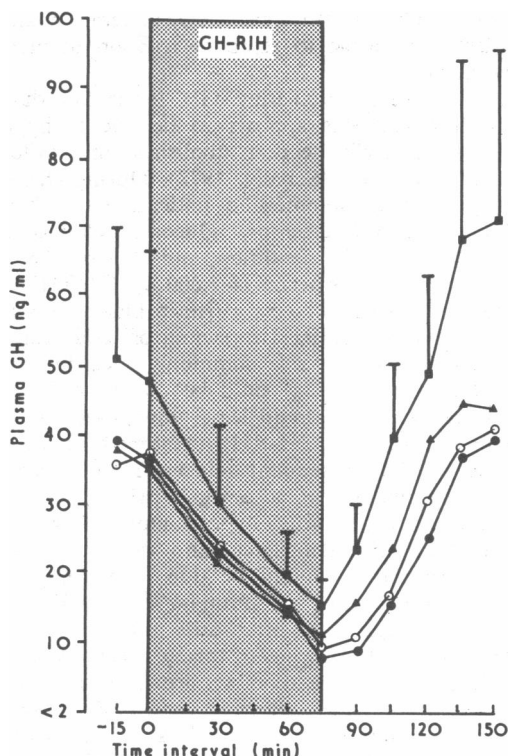


FIG. 1—Mean plasma GH levels in eight acromegalic patients during 75-minute infusions of graduated doses of GHRH (■=100 µg, ●=250 µg, ▲=500 µg, ○=1,000 µg.) For the 100-µg dose mean levels are shown +1 S.E.

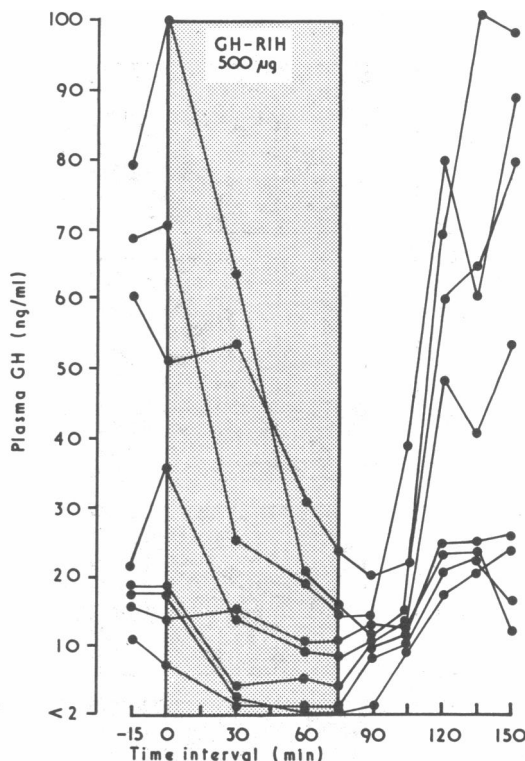


FIG. 2—Individual plasma GH responses to infusions of 500 µg GHRH in eight acromegalic patients.

injections of 500 µg GHRH by any route was one to two hours (table I). When 500 µg was injected with gelatin the time course of action was similar (two hours) though circulating GH levels were reduced to a minimum of 16 ng/ml compared with 22 to 24 ng/ml when GHRH was injected alone by the other routes. The suspension of 4 mg GHRH in arachis oil given by intramuscular injection (table II) resulted in a reduction of GH levels for three to four hours.

TABLE I—Case 3. Plasma GH Levels (ng/ml) in Response to 500 µg GHRH injected Intravenously, Subcutaneously, and Intramuscularly

| Route of GHRH Administration | Time Interval (Minutes): | | | | | | | | | | | |
|---------------------------------|--------------------------|----|----|----|----|----|----|-----|-----|-----|-----|-----|
| | -15 | 0 | 15 | 30 | 45 | 60 | 90 | 120 | 150 | 180 | 210 | 240 |
| Intravenous .. | 34 | 29 | 27 | 25 | 24 | 24 | 26 | 28 | 27 | 30 | 29 | 27 |
| Subcutaneous .. | 35 | 30 | 29 | 26 | 23 | 23 | 35 | 41 | 44 | 32 | 30 | 31 |
| Intramuscular .. | 25 | 24 | 25 | 22 | 24 | 24 | 28 | 47 | 42 | 58 | 42 | 48 |
| Intramuscular in 16% gelatin .. | 29 | 29 | 27 | 20 | 16 | 17 | 20 | 26 | 35 | 24 | 27 | 35 |

TABLE II—Case 5. GH Levels in Response to Intramuscular Injection of 4 mg GHRH suspended in Arachis Oil

| GH levels (ng/ml) | Time Interval (Hours) | | | | | | | | | | | | |
|-------------------|-----------------------|----|----|----|----|----|----|----|----|----|----|----|----|
| | -½ | 0 | ½ | 1 | 1½ | 2 | 2½ | 3 | 4 | 5 | 6 | 7 | 8 |
| .. | 38 | 30 | 14 | 16 | 16 | 15 | 18 | 24 | 32 | 23 | 32 | 24 | 34 |

From 9 to 23 hours GH levels remained between 24 and 35 ng/ml, and at 24 and 30 hours the level was 13 ng/ml.

Effect of Infusion of GHRH on GH and Blood Glucose Responses during Oral Glucose Tolerance Tests.—When the levels of GH were measured during the normal saline infusion after a 50-g oral glucose load each patient showed an increase in circulating GH levels instead of the suppression seen in normal subjects—that is, there was a "paradoxical" rise (fig. 3). When glucose loading was accompanied by the

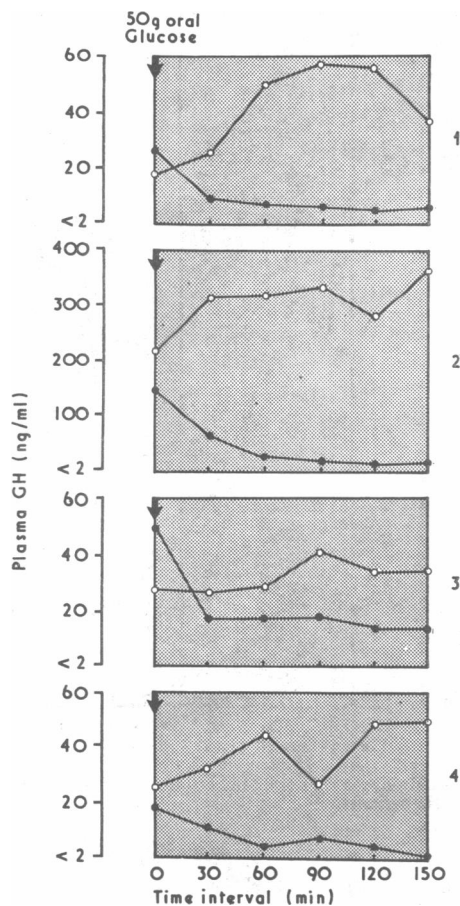


FIG. 3—Cases 1 to 4. GH levels after 50g oral glucose load while receiving infusions of normal saline (O—O) GHRH 1,000 µg (●—●).

infusion of 1,000 µg GHRH over 150 minutes instead of saline the paradoxical rise was prevented and the GH levels were lowered. Study of the effects on blood glucose levels (fig. 4) showed that a normal glucose response was obtained in each patient during the control saline infusion, with peak levels at between 30 and 90 minutes. When the glucose tolerance test was repeated during an infusion of GHRH the results were noticeably different. In cases 2 and 3 there was an initial fall in blood glucose and all four patients showed a delayed rise, so that the maximum level was reached at 150 minutes. Two patients (cases 1 and 4) now had a diabetic type of response, with blood glucose levels of 245 and 165 mg/100 ml at 120 minutes.

Discussion

Since the characterization and subsequent synthesis of GHRH it has been suggested that this cyclic tetradecapeptide may be of use in the treatment of conditions associated with increased circulating levels of GH, such as acromegaly and diabetes mellitus (Hall *et al.*, 1973; Prange Hansen *et al.*, 1973). It was necessary, therefore, to determine the optimum dose which would result in satisfactory inhibition of pituitary GH secretion in acromegalic patients. From our studies doses of 100 to 1,000 µg infused over 75 minutes appeared to be equally effective but a dose of 25 µg was not associated with a consistent response. A dose of 10 µg administered over the same period of time did not produce a significant decrease in GH in any patient. Thus the minimum therapeutic dose for use in acromegaly seems to be in the region of 1.3 µg GHRH per minute, equivalent to 100 µg infused over 75 minutes.

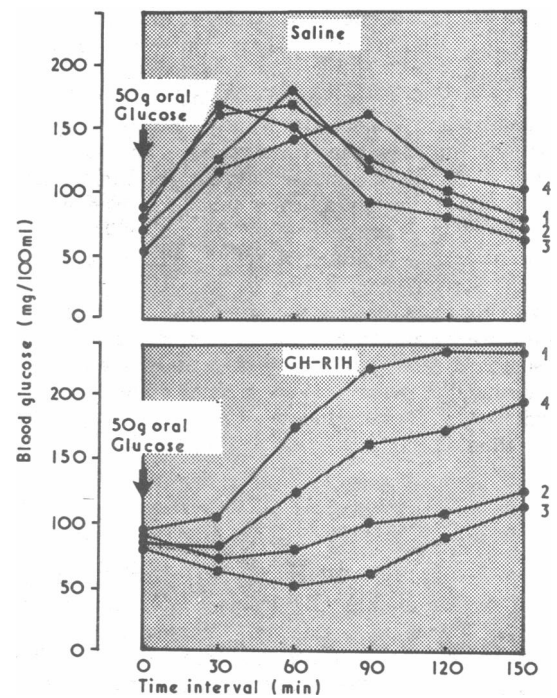


FIG. 4—Cases 1 to 4. Blood glucose responses to 50-g oral glucose load while receiving infusions of normal saline and GHRH 1,000 µg.

Injection of a single dose of 500 µg GHRH by intravenous, subcutaneous, and intramuscular routes caused only slight and transient suppression of GH levels. Though the suspension of 4 mg of the material in arachis oil resulted in a more sustained inhibition of pituitary GH secretion (between three and four hours) the limited duration of action achieved was clearly insufficient for effective therapy. It remains to be seen whether incorporation of GHRH with other repository media, to produce "depot" preparations, will be of any greater value. The possibility of synthesizing analogues of GHRH less susceptible to rapid destruction *in vivo* is also being investigated.

In normal subjects circulating GH levels fall during oral glucose loading and this appears to be due to hypothalamic influences, since stalk section abolishes the influence of hyperglycaemia on GH (Schally, 1972). During these studies we investigated four acromegalic patients who showed the paradoxical rise in GH after oral glucose, well recognized as a common feature of the condition, and showed that this rise was suppressed by the infusion of 1,000 µg GHRH. It seems possible that in normal subjects a rise in blood glucose stimulates the secretion of GHRH to switch off GH secretion and that the paradoxical GH rise commonly seen in acromegaly during oral glucose loading could be due to a deficiency of GHRH secretion. This would leave unopposed the action of GH-releasing hormone (GH-RH) presumed to be secreted in response to the subsequent fall in blood glucose. It is tempting to speculate that acromegaly might be a pituitary manifestation of a primarily hypothalamic disease resulting from isolated FHRH deficiency, and that the development of the pituitary tumour might be due to prolonged and inappropriate stimulation of the pituitary by GH-RH. Pituitary tumours in acromegaly are often still under some degree of hypothalamic control, since in many cases the glucose-sensing mechanisms located there are still able to modify pituitary secretion of GH following changes in blood glucose. The possibility, however, that primary hypersecretion of GH-RH causes acromegaly cannot be excluded. Though these possibilities are intriguing clearly full evaluation of GH secretion can be made only when sensitive assays for GHRH and GH-RH become available. The occasional occurrence of

some suppression of GH levels by a rise in blood glucose in acromegalic patients suggests that there might be only a partial deficiency of GHRH or partially suppressible GH-RH in these patients.

The infusion of GHRH resulted in impairment of glucose tolerance and a change in the shape of the blood glucose curve despite the marked reduction in GH levels. This can be explained by the observations that GHRH impairs insulin secretion in response to glucose by a direct action on the pancreas, as reported in normal subjects by Alberti *et al.* (1973). We have also carried out further studies which confirm this effect. Possibly the reduction in insulin response to glucose and the impairment of secretion of thyroid-stimulating hormone after giving thyrotrophin releasing hormone (Hall *et al.*, 1973) represent non-physiological responses to the large doses of GHRH delivered to the general circulation. Clearly, however, they may have important implications if this hypothalamic hormone is to be used to treat diseases associated with excessive GH secretion. The production of impaired glucose tolerance curves in two patients by GHRH and a rise in the blood glucose levels at 150 minutes

in the other two suggest that the effects of the polypeptide with respect to pancreatic function and the gastrointestinal hormones need to be fully evaluated.

We wish to thank the nursing and technical staff of the Programmed Investigation Unit, Newcastle Royal Victoria Infirmary, and the metabolic ward, St. Bartholomew's Hospital, for their valuable help. The studies have been generously supported by the Joint Research Board of St. Bartholomew's Hospital and the Peel Medical Research Trust.

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Management of Patients with Urinary Calculi

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British Medical Journal, 1974, 1, 355-357

Summary

A retrospective survey was made of 305 patients with proved urinary calculi. When those patients with a solitary stone were compared with those with multiple stones no diagnostically helpful difference was noted in the prevalence of abnormal serum or urine biochemistry, urinary infection, or anatomical abnormality of the urinary tract. The same was true of the stone composition and the need for surgery. It seems that neither routine radiological examination nor regular follow-up is likely to help identify patients whose stones are going to recur.

Introduction

Figures given for the prevalence of urinary calculi vary with the type of survey carried out. Ahlgren and Lorsted (1965), who studied all social groups in a large geographical area, reported the presence of urinary calculi in 80 per 100,000 of the population, whereas Larsen and Philip (1962), in a survey of Danish doctors, found a prevalence rate of 8,719 per 100,000. However, the overall frequency of urinary stones, and hence the work load on stone clinics, does seem to be increasing.

The frequency of recurrence is also variable, some authors finding that 85-90% of patients suffer from solitary stones (Baker and Connolly, 1956; Garvey and Boyce, 1956); whereas Williams (1968) reported that 75% of patients with a urinary stone will form another.

The present survey attempted to define objective criteria whereby patients likely to suffer recurrent calculi could be identified. Radiological examination is commonly used as a routine check in stone clinics to detect unexpected stone formation which may require intervention or further investigation. The value of such examinations has not been clearly shown.

Patients and Methods

A total of 305 patients were included in the survey, which was carried out retrospectively. All the patients had passed a stone or had unequivocal radiological evidence of a urinary calculus and had presented in the 10-year period 1962-71. During the period of this survey no patient was discharged from the clinic and the default rate was less than 2%.

Biochemical investigation of urine and serum is now performed on all patients, though early in the series this was not always done on patients with solitary stones. Serum urea and electrolytes, calcium (three non-occluded venous samples) uric acid, creatinine, and plasma proteins were estimated. Urinary calcium excretion was assessed on three consecutive days on the routine ward diet. Normal ranges were: serum calcium 8.5 to 10.5 mg/100 ml; urine calcium below 300 mg/24 hours. Urine was examined bacteriologically in all cases at the time of presentation and any infection was treated with antibiotics as indicated by culture. Intravenous pyelography was performed on presentation to establish the exact site of the stones and also to detect anatomical abnormalities of the urinary tract. All available stones passed or removed surgically were analysed qualitatively.

Outpatient visits were at three- to 18-month intervals depending on the presence or absence of symptoms, their duration, and the site of the stones. At each visit a clinical appraisal of the symptoms was made and either a plain x-ray film or an intravenous pyelogram was taken. The value of these investigations was assessed by estimating the number of clinically unexpected findings and the treatment indicated by these.

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