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# SOMATOCRININ, GROWTH HORMONE RELEASING FACTOR, STIMULATES SECRETION OF GROWTH HORMONE IN ANESTHETIZED RATS

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Summary. The synthetic replicate of a 44 amino acid peptide isolated from a human pancreatic tumor which had caused acromegaly possesses high specific activity to release growth hormone (GH) in anesthetized male rats. The GH secretion induced by this peptide is dose-dependent from 50 ng to 1 μg, with plasma GH concentrations increasing more than 10-fold within 5 min of iv administration at the higher doses. Two enzymatic degradation products of the 44 residue peptide were also isolated and consist of the first 37 and 40 amino acids. All three peptides appear to possess similar potency, on a molar basis, in vivo, contrary to in vitro results. The specificity of these peptides on GH release was shown by their failure to alter plasma concentrations of prolactin (PRL), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and corticosterone. Based on these in vivo results, the three peptides will serve as powerful tools with which to investigate the mechanisms of GH secretion.

We have recently isolated and characterized a 44 amino acid peptide as well as two of its enzymatic degradation products from a human pancreatic tumor that had caused acromegaly (1). All three peptides possess growth hormone releasing activity and have been designated hpGRF-44, -40, and -37, indicating their source (human pancreas), action (growth hormone releasing factor) and composition (number of amino acids). With the synthetic replicates of these peptides we have been able to investigate their activity in vivo. We report here our initial observations on the dose-response relationship, potency and specificity of hpGRF to stimulate GH secretion in male rats anesthetized with sodium pentobarbital.

ABBREVIATIONS:
hpGRF-44, hpGRF-40 and hpGRF-37: human pancreatic growth hormone releasing factors composed of 44, 40 and 37 amino acids, respectively.

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## MATERIALS & METHODS

Male Sprague-Dawley rats weighing 220-350 g were used in these experiments. Animals were housed in a temperature-(19-22°C) and humidity-controlled vivarium under a 14h light:10h dark lighting schedule (light on at 0600h) and were given food and water ad libitum. Experiments were conducted with groups of 4-6 rats per treatment.

Effect of hpGRF on GH release: Five to twelve min after injection of sodium pentobarbital (NaPB, 60 mg/kg, ip) rats were fitted with a catheter placed in the right external jugular vein and advanced to the superior vena cava. An initial blood sample (0.15 ml) was drawn 15 min after NaPB administration. This was immediately followed by the administration of various doses of the three hpGRF peptides or saline. Subsequent blood samples were drawn as indicated in the figures.

Relative Potency of hpGRFs: To ascertain the relative potency of each peptide, 0.02 nmole of hpGRF-44, -40 and -37 was administered to animals as described above.

Continuous infusion of hpGRF: Rats were fitted with two catheters, one in each external jugular vein. Following two control blood samples (17.5 and 20 min after NaPB) a 30-min continuous infusion of hpGRF-40 (either 20 ng/30µl/min or 500ng/30µl/min) was initiated. At indicated times, blood samples were withdrawn from the other catheter.

Specificity of hpGRF for the release of GH: An initial blood sample was drawn 15 min after NaPB administration, followed by an iv injection of 1 µg of hpGRF-40. Subsequent samples were drawn at 5 and 30 min post-injection through the jugular catheter for measurement of other pituitary hormones and corticosterone.

Hormone assays: Plasma concentrations of pituitary hormones were determined by radioimmunoassay, using the double antibody method, with reagents provided by National Pituitary Agency of the NIH, with the exception of the antisera used in the GH-RIA, which was provided by Dr K. Sinha (2). Samples for PRL, LH, FSH, and TSH were assayed in duplicate using 50-100 µl of plasma. Samples for GH were first diluted 1/5 and then assayed in triplicate using 20-100 µl. Corticosterone was measured as previously described (3), following extraction of samples with methylene chloride.

Peptide preparations: The three peptides were synthesized by solid-phase techniques using a Beckman 990 peptide synthesizer (4). The peptides were dissolved in water and diluted with saline to attain working concentrations.

## RESULTS

Initial studies with hpGRF-40 demonstrated that the maximum GH response occurred within 3-5 min following iv administration, that concentrations declined within 15 min and approached baseline within 30 min. For this reason subsequent studies were limited to the first 15 min following treatment.

As shown in Fig. 1, hpGRF-40 is a potent and fast-acting secretagogue of pituitary GH. The GH response is dose-dependent, reaching a maximum at approximately 1µg. Within the dose-response range, maximum GH release occurred 3-4 min after the bolus iv injection. At higher doses (up to 50 µg,

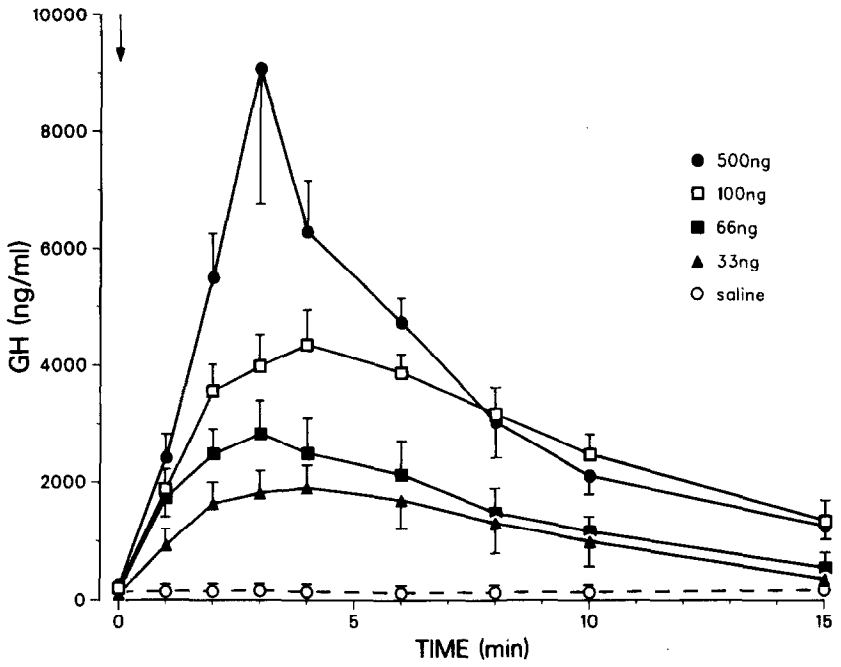


Fig 1: The dose-response relationship of hpGRF-40 on the increase of plasma GH concentrations in anesthetized male rats. Animals were treated with 60 mg/kg sodium pentobarbital (ip) 15 min prior to the initiation of blood-sampling. Immediately after time 0, hpGRF-40 was administered (iv) at the doses indicated in 0.5 ml saline. Data points represent the mean of results obtained in 4 to 6 rats; vertical bars represent SEM.

data not shown), the amplitude of the GH response is not greater than that seen with the 1-μg dose, although it persists slightly longer. These results closely approximate those observed with the other two species of hpGRF.

To compare the potency of hpGRF-44, -40 and -37 we administered a 0.02 nmole dose of each peptide to anesthetized rats. Fig. 2 illustrates that all three peptides are not significantly different in their ability to elicit a release of GH ($p>0.05$, analysis of variance for repeated measures (5)).

The continuous iv infusion for 30 min of a low dose of hpGRF-40 (20 ng/min) resulted in a gradual increase of plasma GH concentrations over 15-20 min, from approximately 100 ng/ml to 600 ng/ml (Fig. 3). These concentrations were maintained throughout the treatment, decreasing only after termination of the infusion. The higher dose of hpGRF-40 (500 ng/min) produced a rapid and dramatic increase in plasma GH concentrations to values between 6-10 μg/ml. This response lasted for approximately 15 min, after which GH concentrations declined in spite of the continued infusion of hpGRF.

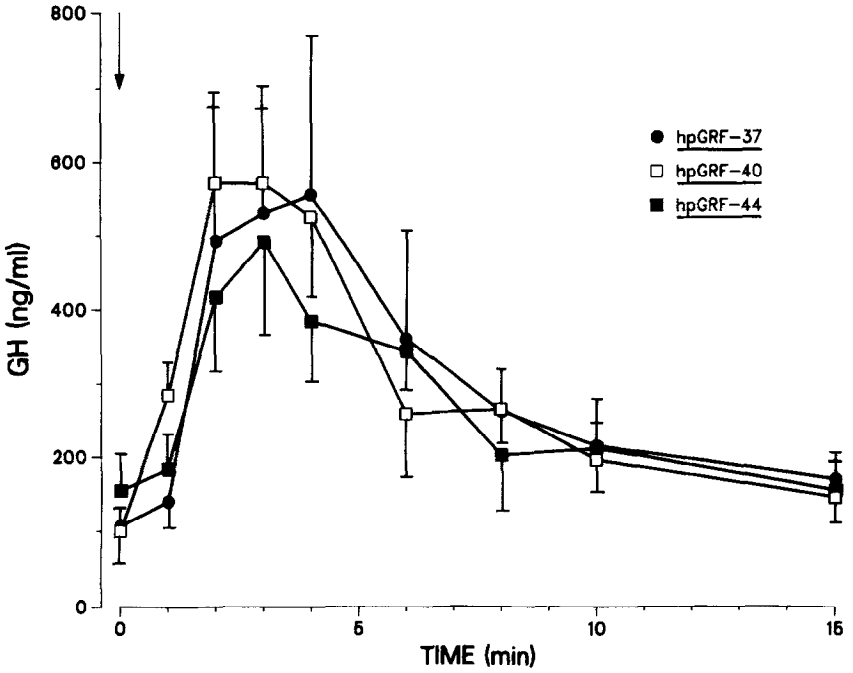


Fig 2: The relative potency of 0.02 nmole hpGRF-44, -40 and -37 in anesthetized male rats. Animals were treated with 60 mg/kg sodium pentobarbital (ip) 15 min prior to the initiation of blood sampling. The peptides were administered (iv) immediately after time 0, in 0.5 ml saline. Data points represent the mean of results obtained in 4 to 6 rats; vertical bars represent SEM.

The iv injection of a maximal dose of hpGRF-40 (1 μg) produced no changes in the plasma concentrations of LH, FSH, TSH, PRL and corticosterone (Table 1).

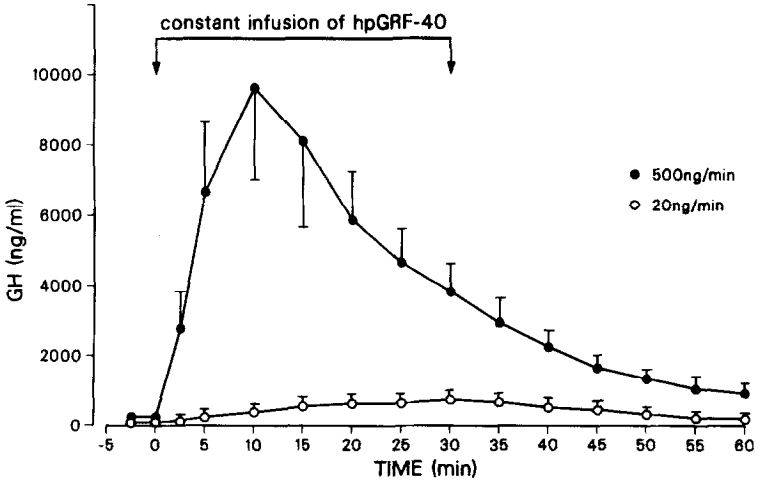


Fig 3: Plasma GH response to a 30-min continuous infusion of hpGRF-40 in anesthetized male rats. hpGRF-40 was infused (iv) at a rate of 500 ng/min (●-●) or 20 ng/min (o-o) beginning 20 min after induction of anesthesia with sodium pentobarbital (60 mg/kg, ip). Data points represent the mean of results obtained in 4 to 6 rats; vertical bars represent SEM.

TABLE 1.

Effect of 1μg hpGRF-40 iv on Plasma Hormone Concentrations in Male Rats Anesthetized with Sodium Pentobarbital

| | LH ng/ml | FSH ng/ml | TSH ng/ml | PRL ng/ml | Corticosterone ng/ml | GH ng/ml |
|---|---|---|---|---|---|---|
| Control | 71 ± 19 | 408 ± 59 | 252 ± 90 | 45 ± 30 | 396 ± 47 | 259 ± 66 |
| 5 min | 73 ± 17 | 386 ± 62 | 274 ± 85 | 42 ± 28 | 413 ± 22 | 5932 ± 1330** |
| 30 min | 86 ± 20 | 381 ± 46 | 293 ± 165 | 30 ± 17 | 523 ± 75 | 1139 ± 628** |

** Significantly different ($p < 0.01$) from control value.

## DISCUSSION

These studies clearly demonstrate the high potency and specificity of the hpGRF peptides in the stimulation of GH secretion *in vivo*. Indeed, the potency of hpGRF is of the same order of magnitude as that observed for other hypothalamic hypophysiotropic regulatory peptides, i.e. thyrotropin releasing factor, gonadotropin releasing factor and corticotropin releasing factor.

Although the amidated form of hpGRF-44 is apparently the parent molecule and possesses the highest activity *in vitro* (1), all three peptides are virtually equipotent *in vivo*. The differences in potency between the *in vitro* and *in vivo* systems may be due to the reduced ability of the *in vivo* system to discern such differences because of its inherent variability or the peptides may undergo proteolytic processing *in vivo* to yield a common bioactive molecule. Other studies we have conducted have shown that deletion of the N-terminal amino acid, tyrosine, from hpGRF results in complete loss of bioactivity, indicating that the active core starts with the first N-terminal amino acid (1).

The NaPb-treated animal is a useful model to study the effects of hpGRF. Administration of NaPb results in an increase in plasma GH concentrations (6). The mechanism of this response has not been clarified, but the amplitude of the GH increase is similar to the increase in GH concentrations observed in rats treated with antibodies against somatostatin (7). Thus it is possible that the elevation of plasma GH following injection of NaPb is due to the inhibition by the drug of endogenous somatostatin secretion. Likewise, it appears that NaPB treatment also interrupts the endogenous release of hypothalamic GRF since we did not observe any spontaneous pulses of GH in over 40 anesthetized animals studied during the time interval when conscious rats are

known to have spontaneous GH pulses (8). Thus with this model we are effectively able to interrupt the hypothalamic dual-control system regulating GH secretion and can investigate the effects of hpGRF unencumbered.

The amount of GH which can be released by the rat pituitary under a constant hpGRF stimulus is remarkable. The average plasma GH concentration during the 30-min continuous infusion of hpGRF (500 ng/min) was 6483 ± 791 ng/ml. Assuming a total blood volume of 15 ml in these animals, one can estimate that GH released by the pituitary must be at least in the range of 100 μg (15 ml X 6 μg/ml) or even higher considering extracellular distribution and degradation. This value approximates pituitary content of GH in the rat (9). It remains to be clarified whether the decline in GH concentration observed in rats during the continuous administration of the high dose of hpGRF-40 is due to pituitary depletion of GH or to a down-regulation of GRF receptors. At lower concentrations of hpGRF (20 ng/min), GH concentrations are maintained at a constant value, which in more recent experiments has lasted as long as 60 min. The immediate increase and very rapid decrease of plasma GH concentrations after hpGRF administration indicates that hpGRF has a very short biological half-life, though this has yet to be determined.

## ACKNOWLEDGEMENTS

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