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THE EFFECTS OF LONG TERM GROWTH HORMONE RELEASING FACTOR (GRF 1-40) ADMINISTRATION ON GROWTH HORMONE SECRETION AND SYNTHESIS IN VITRO

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Summary: The ability of human pancreatic GH releasing factor 1-40 (hpGRF 1-40) to release GH has been studied in rat anterior pituitary cells in primary culture. Over 24 hours hpGRF (1-40) increased total (cell content and secretion) production 2-fold with an ED_{50} of 20 pM. Subseqent hpGRF (1-40) stimulation of GH release was not affected by pretreatment when the fall in stored GH was taken into account. In contrast LH responses to gonadotrophin releasing hormone (GnRH) were markedly desensitized after 24 hours GnRH pretreatment in the same experimental system and using the same analysis. hpGRF (1-40) responses were not desensitized when pretreatment was for 3, 12 or 24 hours. The data show that hpGRF (1-40) responses do not desensitize in our experimental conditions under which GnRH responses show marked desensitization.

Recently several potent GH releasing peptides have been isolated from human pancreatic tumours and characterised (1-3). These peptides are similar to rat hypothalamic GH releasing hormone (4) and may be the same as human hypothalamic GH releasing hormone (4,5). It is well recognised that in a number of biological systems prolonged treatment with an agonist leads to a lower subseqent response to that agonist (6-14). This desensitization may occur as a result of decreased number or affinity of specific receptors for that agonist but post receptor events have also been implicated. The desensitization phenomenon has been convincingly demonstrated in the context of the LH response to the hypothalamic neuropeptide GnRH in vivo (6,11-14) and in vitro (7,8) and GnRH is secreted in a pulsatile manner by the hypothalamus

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Abbreviations used: GH; growth hormone, LH; luteinizing hormone, hpGRF (1-40) human pancreatic GH-releasing factor 1-40, GnRH; gonadotropin-releasing hormone.

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(6,11). These data form the basis of the rationale that underlies the present therapeutic approach of pulsatile GnRH administration (6,11).

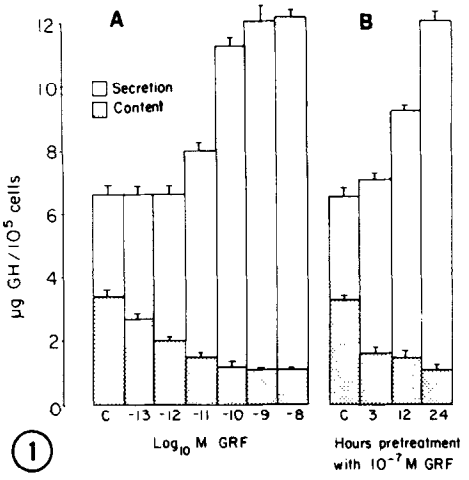
In this study we have investigated the effects of hpGRF (1-40) on the secretion of GH from primary cultures of male rat anterior pituitary (AP) cells after pretreatment with hpGRF (1-40) at different concentrations and from 3-24 hours.

MATERIALS AND METHODS

Materials: The effect of hpGRF (1-40) (purchased from Cambrige Research Biologicals, Cambridge, England) on GH production by 4 day primary cultures of adult (180-220g) male Wistar rat AP cells was studied. Cell cultures were prepared as described previously (15). In brief, cells were isolated from AP glands using a collagenase based method and were plated at a density of approximately 10^5/ml/well. Linbro 96 well multiwell plates were used, each well having a growth area of 2.3 cm^2 (Flow Laboratories, Rockville, MD). The culture medium was semi-synthetic as described by Brazeau et al[3]. hpGRF (1-40) was stored in 2N acetic acid and diluted in saline. GnRH (Hoechst, Hounslow, England) was dissolved in saline and A23187 (Boeringer Mannheim, New York, NY) was dissolved in dimethyl sulphoxide (Sigma, St. Louis, MO) and then saline. Before incubation with hpGRF (1-40) the cells were washed twice with balanced salts solution (Earles, Gibco Europe, Paisley, Scotland). Subsequent incubation with hpGRF (1-40) was in semi-synthetic medium. GH and LH were measured using materials kindly provided by Dr. A.F. Parlow and the NIADDK (Bethesda, MD). GH and LH cell contents were measured after the cells had been removed from the culture plates and lysed by incubation in Ca^{2+} and Mg^{2+} free balanced salts solution with 0.1N NaOH for 30 minutes. ED_{50} values were determined by log probit transformation and subsequent linear regression analysis. Significance was determined by multiple t test.

RESULTS

Pretreatment with hpGRF (1-40) over a 24 hour period produced a fall in cell GH content and an increase in medium GH content (Fig. 1A). Overall the total GH produced by the cells doubled with maximum doses of hpGRF (1-40) over a 24 hour period with an ED_{50} of 0.02 nM. Pretreated cells were then subject to further stimulation with either hpGRF (1-40) or the Ca^{2+} channel ionophore A23187 (which acts as a maximal and non-specific stimulus to secretion) over a 6 hour period (Fig. 2). The amount of GH released fell in proportion to the dose of hpGRF (1-40) used to pretreat the cells. hpGRF (1-40) responses were significantly better after each pretreatment than those induced by A23187. The greater the concentration of hpGRF (1-40) used to pretreat the cells the greater the disparity between GH responses to the two



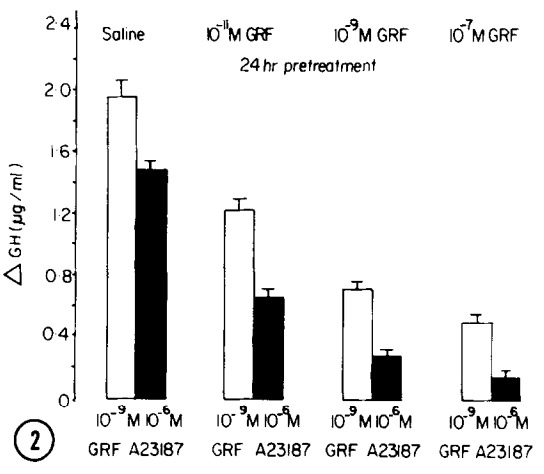


Fig. 1A. Cell (stippled portion) and medium (open portion) GH content after 24 hours incubation with increasing concentrations of hpGRF (1-40). Cell GH content fell whereas GH produced by the cultures doubled at the highest doses of hpGRF (1-40) used. At low doses hpGRF (1-40) released GH from the cells without increasing total GH. Figure 1B shows cell and medium GH in response to 10^{-7}M hpGRF (1-40) over 3, 12 and 24 hour periods. Controls at time zero and at 24 hours did not differ by more than 20%. The results shown are typical of four experiments each performed with not less than 9 replicates per point. Values are means ± SEM.

Fig. 2. The release of GH by 10^{-9}M hpGRF (1-40) or the ionophore A23187 over a 6 hour period after pretreatment with different doses of hpGRF 1-40 for 24 hours. The previous 24 hour incubation medium had been removed and the cells washed twice. Appropriate vehicle controls were included. Data were typical of at least 3 separate experiments each performed with not less than 9 replicates per point.

stimuli. Figure 3A shows the amount of GH released corrected to account for the depletion of GH content due to the pretreatments. This was achieved by multiplication of the amount of GH released by the ratio of cell GH content in saline pretreated cells to cell GH content in hpGRF (1-40) pretreated cells. This form of analysis demonstrated desensitization of the LH response to GnRH (7) (Fig. 4). However, the ED_{50}'s and maximum responses of hpGRF (1-40) dose response curves analysed in this way were not significantly different from those obtained from cells pretreated with saline alone. The fall in cell GH content with hpGRF (1-40) pretreatment was not as marked as the fall in A23187 releasable GH (Fig. 3B). Thus cell GH content overestimates the pool of GH available for release by a non specific stimulus. (Similar data were obtained using K^+ depolarisation as non specific stimulus).

When shorter periods of hpGRF (1-40) pretreatment were used subsequent hpGRF (1-40) dose response curves also remained similar to control values

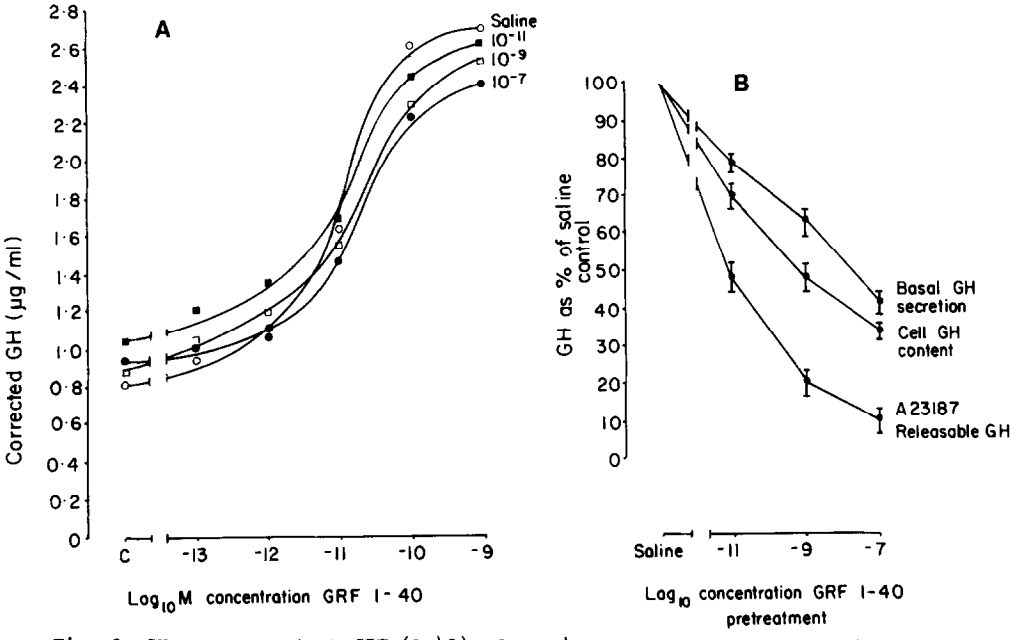


Fig. 3 GH responses to hpGRF (1-40) after 24 hour pretreatments are illustrated in figure 3A. The responses were determined over a 6 hour period after the previous 24 hour preincubation with hpGRF (1-40). The amount of GH released has been corrected for the amount of GH available for release within the cells (see text). Expressed in this way the curves are statistically identical. Figure 3B shows the percentage fall in basal GH secretion, cell GH content and ionophore A23187 releasable GH, relative to saline control, in each of the hpGRF (1-40) pretreated groups. All data were typical of at least 3 separate experiments each performed with not less than 9 replicates per point.

when analysed in proportion to cell GH content (Fig. 5A). The small, but significant reduction in ED_{50} that occurs after 3 and 12 hour pretreatments with an excess (10^{-7}M) of hpGRF (1-40) probably indicates a saturation of receptors/cellular pathways rather than a desensitization effect. The changes in the affinity of the GH response to GnRH after GnRH pretreatment have been reported as over 20 fold (7) and more than 2 orders of magnitude in this study (Fig. 4). The ionophore A23187 consistently released less GH than hpGRF (1-4) at each time point. Over a 6 hour period A23187 did not increase total GH production in contrast to hpGRF (1-40) (data not shown).

DISCUSSION

In this study we have evaluated GH responses to hpGRF (1-40) after prior, long term, stimulation with the releasing factor to search for evidence of desensitization *in vitro*. Although hpGRF (1-40) shows only 67% homology with

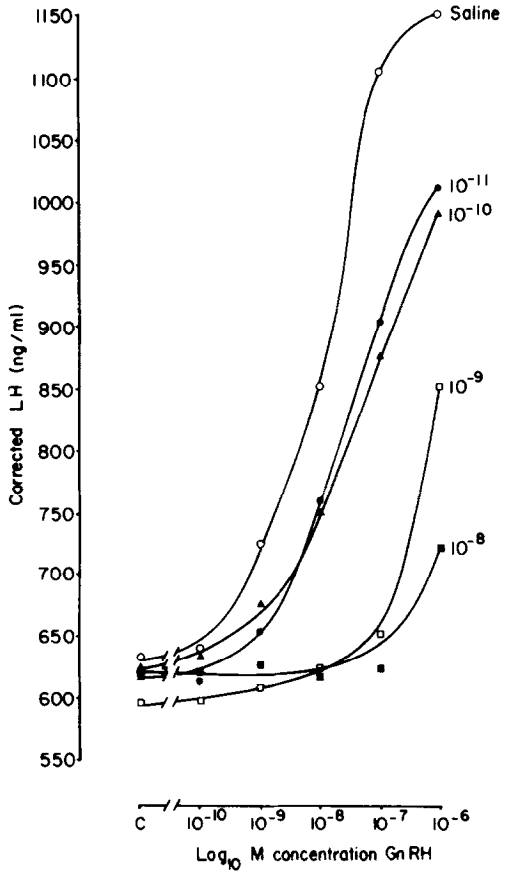


Fig. 4 LH secretion over a 6 hour period in response to GnRH and after 24 hours pretreatment with either saline or GnRH at differing concentrations. SEM are omitted for clarity but represent less than 8% of the mean value (n = 9). Data were analysed as described for figure 3. Results are typical of 3 separate experiments.

rat hypothalamic GRF (4) available evidence suggests that GRF peptides stimulate GH secretion via the same pathway (1-4). Treatment with hpGRF (1-40) over a 24 hour period doubled the amount of GH produced by the cultures in a dose-dependent manner (Ed_{50} 0.02 nM), an observation that probably indicates de novo GH synthesis (15,16) subsequent to increased mRNA levels (7) although our own studies are not definitive. If hpGRF (1-40) responses after prior hpGRF (1-40) treatment were corrected for the size of the GH pool available, the magnitude the ED_{50} of each subsequent response were similar to saline pretreated cells GnRH. These findings contrast with those obtained with GnRH stimulated LH release in the same experimental system. Desensitization of GnRH responses *in vitro* (7,8) and *in vivo* (6,11-14) is an established

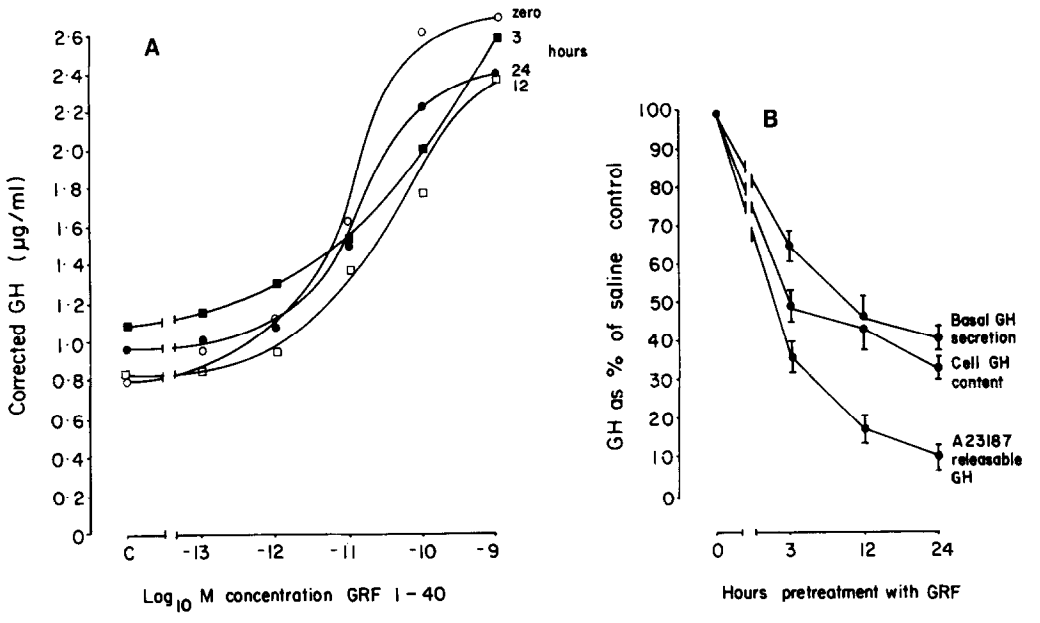


Fig. 5 GH dose response curves to hpGRF (1-40) constructed after pretreatment with 10^{-7}M hpGRF (1-40) for 3, 12 and 24 hours. Experimental protocol was otherwise as described above. Data are again multiplied by the ratio of cell GH content without pretreatment to that after pretreatment. The ED_{50}'s for the curves were 0.01 nM (zero), 0.06 nM (3 hours), 0.05 nM (12 hours) and 0.02 nM (24 hours). The 3 and 12 hour ED_{50}'s were slightly but significantly lower than control ($p < 0.05$ $n = 3$) whereas 24 hours after treatment the ED_{50} was unchanged. Fig. 4B illustrates percentage fall in basal GH secretion, cell GH content and ionophore induced GH release from parallel cell cultures.

phenomenon. One must be cautious in interpreting the results of *in vitro* studies especially as it is likely that GRF peptide (18,19) and other GRF entities (eg. epinephrine (20) and acetylcholine (21)) as well as the inhibitor somatostatin (22) are released in multiple pulses. However it is clear that in the *in vitro* model used in this study somatotroph responses to hpGRF (1-40) differ markedly from gonadotroph responses to GnRH. GRF peptides are linked to GH release by adenyl cyclase (23), a characteristic that is frequently associated with desensitization and further studies are required to determine whether desensitization occurs after longer periods of GRF treatment, when GRF is administered continually (subject to its degradation) and when GRF is given in combination with other neuroregulators of GH secretion.

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