Effects of growth hormone-releasing factor, somatostatin and dopamine on growth hormone and prolactin secretion from cultured ovine pituitary cells

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A synthetic form of human pancreatic growth hormone releasing factor (GRF-44-NH_2) was shown to be a potent stimulator of growth hormone (GH) secretion and cellular cyclic AMP levels in cultured sheep pituitary cells. A small dose-dependent stimulation of prolactin secretion was also observed. Somatostatin (0.5 μM) completely blocked the maximal GRF (1 nM)-stimulated secretion without a significant effect on cyclic AMP levels. Dopamine (0.1 μM) inhibited the GRF-elevated GH secretion by 50% and lowered cyclic AMP levels by 30%. Dopamine (0.1 μM) inhibition of basal prolactin secretion was not affected by GRF (1 nM). The data support the hypothesis that cyclic AMP is involved in the action of GRF but suggest that somatostatin can inhibit GRF-induced secretion of GH independently of cyclic AMP.

Growth hormone releasing factor *Growth hormone* *Prolactin* *Somatostatin* *Dopamine* *Cyclic AMP*

1. INTRODUCTION

Secretion of GH by the anterior pituitary gland is under the control of stimulatory and inhibitory factors produced by the hypothalamus. The major inhibitory factor, somatostatin, has been shown to exist in at least two active forms [1], SRIF-14 and the N-terminally extended SRIF-28. Two releasing factors (GRFs) have been isolated and characterised from human pancreatic islet cell tumours [2,3]. The larger and more potent of these peptides (44 residues) has been shown to be chromatographically similar to a partially purified rat hypothalamic GRF. Both rat and human GRFs stimulated GH secretion in vitro by cultured rat pituitary cell monolayers and the dose-response curves were parallel [2]. A rat hypothalamic GRF has recently been more fully characterised; it shows 67% homology with human GRF (44-NH_2) and has very similar effects in the rat pituitary cell monolayer system [4].

We describe here the effects of the most potent ectopic human GRF, the 44-NH_2 residue polypeptide, on sheep anterior pituitary cells in primary culture. We previously observed that both SRIF and DA can inhibit GH and prolactin secretion from sheep pituitary cells stimulated by the phosphodiesterase inhibitor isobutylmethylxanthine [5] and we have therefore investigated also the effects of these inhibitory hypothalamic factors on secretion induced by the more specific secretagogue, GRF.

Abbreviations: GRF, growth hormone releasing factor; SRIF, somatostatin; DA, dopamine; GH, growth hormone; VIP, vasoactive intestinal peptide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid

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2. MATERIALS AND METHODS

2.1. *Materials*

Synthetic GRF (44-NH_2) and SRIF were obtained from Universal Biologicals, Cambridge. DA was purchased from Sigma, Poole. Sera and culture media were obtained from Gibco, Paisley. Ovine GH (NIH-GH-S9; 0.56 IU/mg) and ovine prolactin (NIAMDD-oPRL-14; 31.0 IU/mg) were gifts from Dr A.E. Wilhelmi and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda.

2.2. *Preparation and culture of pituitary cells*

Cells were prepared and incubated as in [6]. Ovine anterior pituitary cells were dispersed (90 min, 37°C) using collagenase (Boehringer, 1.5 mg/ml), hyaluronidase (Sigma-type ls, 0.5 mg/ml), deoxyribonuclease I (Boehringer, grade II; 0.25 mg/ml) and bovine serum albumin (Sigma fraction V; 30 mg/ml) in medium containing NaCl (137 mM), KCl (5 mM), Na_2HPO_4 (0.7 mM), glucose (10 mM) and Hepes (25 mM), which was adjusted to pH 7.4 with NaOH. Cells were collected by centrifugation ($400 \times g$ for 2 min) and washed 5 times by resuspension in the Hepes-buffered medium and centrifugation. The cells were pipetted ($0.5–1.5 \times 10^6$ cells/dish) into 3.5-cm culture plates (Sterilin), and incubated in Dulbecco's modified Eagle medium (3 ml/dish) containing 25 mM Hepes, 5% foetal calf serum, 10% horse serum and antibiotics for 72 h at 37°C under 95% air/5% CO_2.

2.3. *Experimental incubations*

After 72 h the incubation media were discarded and the cells were washed 3 times with the incubation medium without serum. The cells were preincubated for 30 min, and then the media were replaced and experimental incubations were initiated by the addition of test substances. At the end of a further 30 min incubation the media were centrifuged ($400 \times g$, 2 min), and the supernatants stored at −20°C until assayed for hormone content. The cellular cyclic nucleotides were extracted with trichloroacetic acid [10%(w/v) 2 h at 4°C] and stored frozen at −20°C, prior to assay.

2.4. *Assays*

The radioimmunoassay procedures for both ovine GH and prolactin were based on methods using specific antisera for these hormones raised in rabbits as in [6]. The cross-reactivities of prolactin and GH in these assays were less than 1%. Hormones were iodinated with $NA^{125}I$ using a modification of the method in [7] as in [6]. All samples, standards etc. were dissolved in assay buffer containing sodium phosphate (0.05 M), pH 7.6, merthiolate (0.6 mM), bovine serum albumin (0.05%) and Triton X-100 (0.05%). Cellular cyclic AMP samples were acetylated and measured as in [8].

3. RESULTS

3.1. *Effect of GRF on secretion of GH and prolactin*

The effects of various GRF concentrations on GH and prolactin secretion after 30 min are shown in fig.1. GH secretion was maximally stimulated by 1 nM GRF to nearly 4-times the basal secretion level. The lowest dose of GRF tested (1 pM) stimulated GH secretion by 50%. Half-maximal stimulation occurred at about 8 pM. The effect of 1 nM GRF on GH secretion was significant at the earliest time tested, 2 min (50% stimulation, $p < 0.01$). GRF also produced a small but significant dose-dependent stimulation of prolactin secretion, to 20–25% over the basal level at 1 nM, GRF.

3.2. *Effect of GRF on cellular cyclic nucleotide concentrations*

GRF stimulated cellular cyclic AMP concentrations in a dose-dependent manner with a significant 2.5-fold increase at 10 pM GRF after 30 min. A maximal 10–12-fold stimulation was achieved at 1 nM GRF (fig.1). Cyclic AMP concentrations were significantly elevated (4-fold) by 1 nM GRF at 2 min. There was no significant change detected in basal cyclic GMP cellular content at any GRF dose tested (not shown).

3.3. *The effects of somatostatin and dopamine on GRF-stimulated hormone secretions*

SRIF (0.5 μM) completely blocked the 5-fold stimulation of GH secretion induced by 1 nM GRF. SRIF alone inhibited basal GH secretion by 30% (fig.2). DA (0.1 μM) partially inhibited the effect of GRF by 50% but had no significant effect

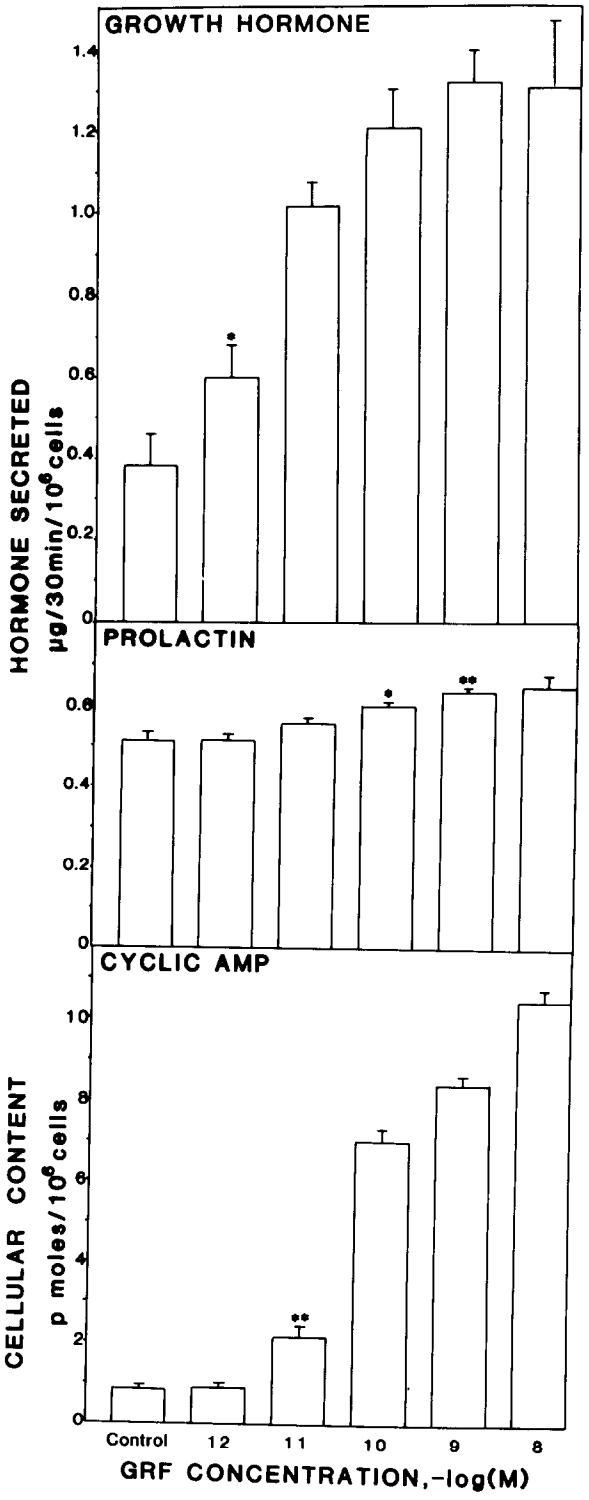

Fig.1. Dose-response relationships for GRF stimulation of GH secretion (top), prolactin secretion (middle) and cellular cyclic AMP levels (bottom). Sheep pituitary cells were cultured for 3 days then exposed to GRF at the concentrations indicated for 30 min. For each treatment the values for hormone released and cyclic AMP content represent the mean values ± SE/30 min per 10^6 cells obtained from 5 replicate dishes. Control and experimental (+GRF) values were compared for statistical differences by Student's *t*-test. Probability values: $*p<0.05$, $**p<0.01$.

on basal GH secretion (fig.2). On the other hand, the stimulation of prolactin secretion by GRF was completely blocked by both DA and SRIF. DA alone inhibited basal prolactin secretion by 40% whilst SRIF had no significant effect (fig.2).

3.4. *Effects of somatostatin and dopamine on GRF-elevated cyclic AMP concentrations*

SRIF had no significant effect on the 8-fold increase in cellular cyclic AMP content induced by GRF (fig.2). DA inhibited the effect of GRF on cyclic AMP concentrations by a third (fig.2). SRIF and DA both showed small (25%) inhibitions of basal cyclic AMP levels but only the effect of SRIF was statistically significant.

4. DISCUSSION

Our data demonstrate that the human pancreatic tumour-derived GRF (44-NH_2) has a rapid and potent stimulatory effect on release of GH from cultured sheep pituitary cells. Half-maximal stimulation of GH secretion occurred at a concentration of 8 pM GRF, close to that found in studies using cultured rat pituitary cells (15 pM [2,9]). The rapid rise in secretory activity observed following addition of GRF to sheep pituitary cells in vitro is consistent with the time course reported for stimulation of GH secretion from perifused rat cells [9] and a study of secretion in the anaesthetised rat [10]. These results suggest that a molecule similar to the human GRF may be involved in the physiological regulation of GH secretion in the sheep.

It has been reported previously that GRF had no effect on prolactin secretion in vitro [2], though a small effect has been observed [9]. We observed a significant effect on prolactin secretion but only at a concentration of GRF (100 pM) 100-fold greater than the minimum required to stimulate GH secretion. This apparent lack of specificity might be due

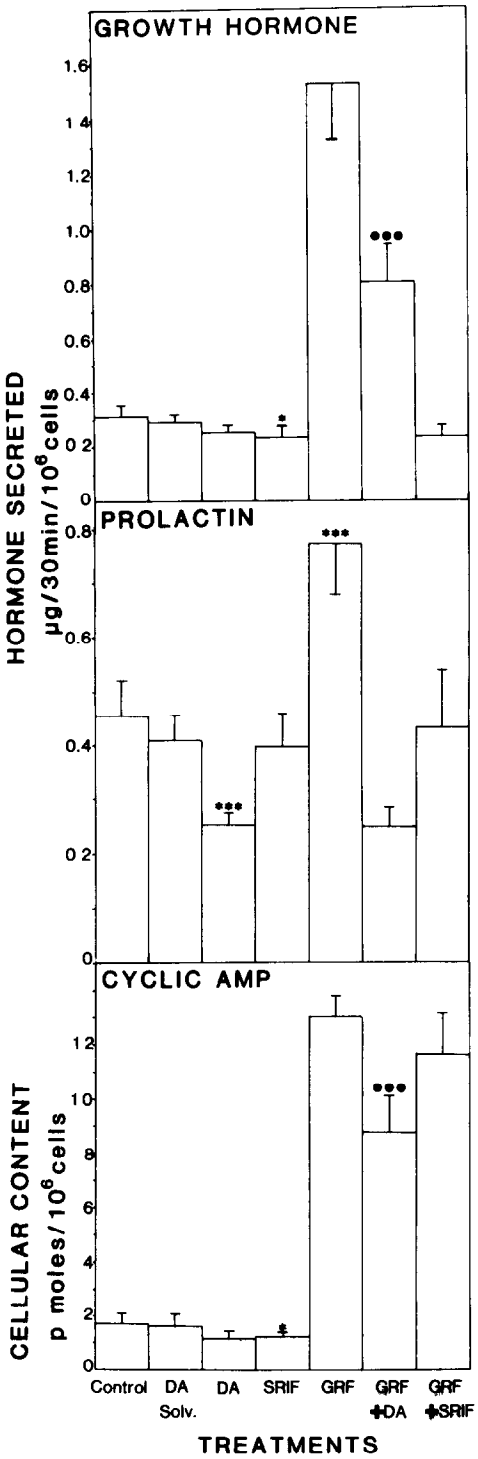

Fig.2. Effects of GRF (1 nM), SRIF (0.5 μM) and DA (0.1 μM) on GH secretion (top), prolactin secretion (middle) and cellular cyclic AMP content (bottom). The histograms represent mean values ± SE/30 min per 10^6 cells. Data were combined from 5 experiments each using 4 dishes of cells per treatment and values were compared for significant differences by Student's t-test. Significance levels: $*p<0.05$, $***p<0.001$ compared with the appropriate controls; $\bullet\bullet\bullet p<0.001$ compared with GRF alone. DA solv: control in which the antioxidant buffer used to dissolve DA [6] was added to the incubation.

to the presence of a few GRF receptors on lactotrophs. Alternatively, since GRF has been shown to interact with VIP receptors [11], it is possible that the stimulatory effect may be mediated via such receptors located on pituitary lactotrophs (VIP and GRF are homologous peptides). Other explanations are also possible.

GRF-stimulated GH secretion was completely blocked by SRIF but only partially inhibited by DA, while the stimulation of prolactin secretion was reversed by both DA and SRIF. These results accord with our earlier work showing that DA inhibited isobutylmethylxanthine-stimulated GH secretion from sheep pituitary cells [5] and suggest that somatotrophs may possess inhibitory DA receptors. On the other hand, DA inhibition of GRF-stimulated GH secretion was not seen in the rat [12]. Whether this represents a species difference or the fact that a crude tumour medium which contained GRF was used in the experiments on rat pituitaries remains to be determined. Our finding that SRIF inhibited GRF-induced prolac tin secretion from sheep pituitary cells is consistent with recent reports that SRIF inhibits prolactin secretion stimulated by thyrotropin releasing factor and VIP in the rat [13,14].

The rapid and extensive elevation of cellular cyclic AMP concentrations induced by GRF coincided with the increase in secretory activity. This supports the concept [15] that cyclic AMP is involved in mediating intracellularly the response to the releasing factor. Inhibition of GRF-stimulated GH secretion by SRIF appeared to be independent of the changes in cyclic AMP induced by GRF (fig.2), which suggests that the major site of action of SRIF is beyond cyclic AMP production, as concluded previously [15,16]. These findings differ from those of authors in [17] who showed a dose-related inhibition of GRF-stimulated cyclic AMP levels by SRIF using rat pituitary cells and an incubation period of 3 h. One possible reason for

this discrepancy may be that different incubation periods were used in the two studies. We found that GRF-induced cyclic AMP concentration reached a maximum within 15 min and then decreased, despite the continued presence of GRF. Effects of SRIF measured at 30 min and 3 h might well be expected to differ. It is possible therefore that the initial rapid stimulation of cyclic AMP by GRF is not blocked by SRIF while during prolonged exposure the lowering of stimulated cyclic AMP levels could become more important. A more detailed investigation of the time course for inhibition is required to resolve this question.

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