Effects of growth hormone-releasing factor and somatostatin on growth hormone secretion and cellular cyclic AMP levels

Cultured ovine and rat anterior pituitary cells show markedly different responses

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Human pancreatic growth hormone-releasing factor (GRF-44-NH_2) stimulated growth hormone (GH) secretion and intracellular cyclic AMP levels in cultured pituitary cells from both sheep and rat. Somatostatin (SRIF), over a wide range of doses and time, showed no significant effect on the elevated cyclic AMP levels in sheep cells, but did block the GH release in a dose-dependent manner. In rat cells, however, SRIF inhibited GRF-stimulated cyclic AMP levels by 75% maximum (still 8-fold greater than the basal levels) and GH release to almost half the basal value. We conclude that somatostatin inhibits GRF-elevated cyclic AMP levels in rat pituitary cells but not in sheep cells.

Growth hormone-releasing factor *Growth hormone* *Somatostatin* *Cyclic AMP* *Rat pituitary cell* *Sheep pituitary cell*

1. INTRODUCTION

The secretion of growth hormone (GH) by the anterior pituitary gland is controlled by at least two hypothalamic factors, a stimulatory GH-releasing factor (GRF) and an inhibitory factor, somatostatin (SRIF). A role of cyclic AMP in the control of GH secretion was first suggested by the stimulatory effects of inhibitors of cyclic nucleotide-phosphodiesterase [1,2] and cyclic AMP derivatives [3] on GH release in vitro. Further support for the role of this nucleotide in the regulation of GH secretion was provided by the inhibitory effects of SRIF on cyclic AMP levels as well as on GH secretion [4–6]. However, other data suggest that the primary site of action of SRIF is at a step after the elevation of cyclic AMP [7,8]. Recently, peptides with GH-releasing activity have been identified [9,10] and shown to stimulate cyclic AMP levels in cultured pituitary cells from rat [11,12] and sheep [13]. Furthermore, the human pancreatic GRF (1-40)-NH_2 characterised in [10] has been reported to stimulate adenylate cyclase activity in a membrane fraction from rat pituitary cells [14]. On the other hand, studies of the action of SRIF on GRF-stimulated cyclic AMP levels in pituitary cells have produced variable results [11–13].

Our previous observations showed no significant inhibition of GRF-elevated cyclic AMP levels by SRIF in sheep pituitary cells [13], whereas in rat cells, a 75% inhibition has been reported [11,12]. To clarify the basis for this difference, which is of considerable potential interest for studies of the mechanism of action of SRIF, we have investigated the effects of SRIF and GRF (44-NH_2) on both rat and sheep cells in parallel.

Abbreviations: GRF, growth hormone-releasing factor; SRIF, somatostatin; GH, growth hormone

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2. MATERIALS AND METHODS

2.1. *Materials*

Synthetic GRF (44-NH_2) and SRIF were obtained from Universal Biologicals, Cambridge, England. Sera and culture media were obtained from Gibco Biocult, Paisley, Scotland. Ovine GH (NIH-GH-S9, 0.56 IU/mg) and rat GH (NIH-GH-B6, 1.7 IU/mg) were gifts from Dr A.E. Wilhelmi and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, USA.

2.2. *Preparation and culture of pituitary cells*

Cells were prepared and incubated according to [15]. Anterior pituitary glands from (4–8-month-old) male sheep and (200–250 g) male Sprague-Dawley rats were dispersed (90 min, 37°C) using collagenase (Boehringer, 1.5 mg/ml), hyaluronidase (Sigma-type 1S, 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 × *g* for 2 min) and washed 5 times by resuspension in the Hepes-buffered medium and centrifugation. Dispersed ovine cells were pipetted (0.5–1.0 × 10^6 cells/dish) into 3.5 cm culture plates (Sterilin), and incubated in Dulbecco's modified Eagle medium (3 ml/dish) containing 5% foetal calf serum, 10% horse serum and antibiotics for 72 h at 37°C under 95% air/5% CO_2 [15]. Dispersed rat cells were plated (0.4 × 10^6 cells/well) into Nunclon (6 × 4) multiwell dishes (Gibco Biocult) and incubated under the same conditions as the ovine cells but with only 1.5 ml/well culture medium.

2.3. *Experimental incubations*

After 72 h the incubation medium was discarded and the cells were washed 3 times with incubation medium from which serum was omitted. The cells were preincubated for 60 min with serum-free medium, and then the medium was replaced and experimental incubations were initiated by the addition of test substances. At the end of the incubation time the media were centrifuged (400 × *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 extraction and assay.

2.4. *Assays*

The radioimmunoassay procedures for both ovine GH and rat GH were based on previous methods [15]. Hormones were iodinated with $Na^{125}I$ by the iodogen method in [16]. 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 according to [17].

2.5. *Treatment of results*

All experiments were performed at least twice with different preparations of pituitary cells and consistent results were obtained. In each case the results of a representative experiment have been presented. Control and experimental values were compared for statistically significant differences using Student's *t*-test.

3. RESULTS

3.1. *Time course of the effect of GRF on GH secretion and its inhibition by SRIF: sheep pituitary cells*

The stimulation of GH secretion from sheep pituitary cells by 1 nM GRF (fig.1) was significant at the earliest time tested, 2 min (80% stimulation, $p < 0.001$). The maximum stimulation of GH release lasted for 5 min, after which a lower (but still elevated) secretion rate for GH was maintained throughout the time course. By 30 min total GH secretion was 4–5-fold greater than basal (and maximum stimulation of secretion rate was considerably greater than this; fig.1). SRIF (0.5 μM) completely blocked this stimulation during the first 15 min, and after 30 min incubation SRIF had inhibited GRF-elevated GH secretion to below the basal secretion rate.

3.2. *Time course of the effect of GRF on cellular cyclic AMP levels and its inhibition by somatostatin in sheep pituitary cells*

GRF (1 nM) stimulated cellular cyclic AMP

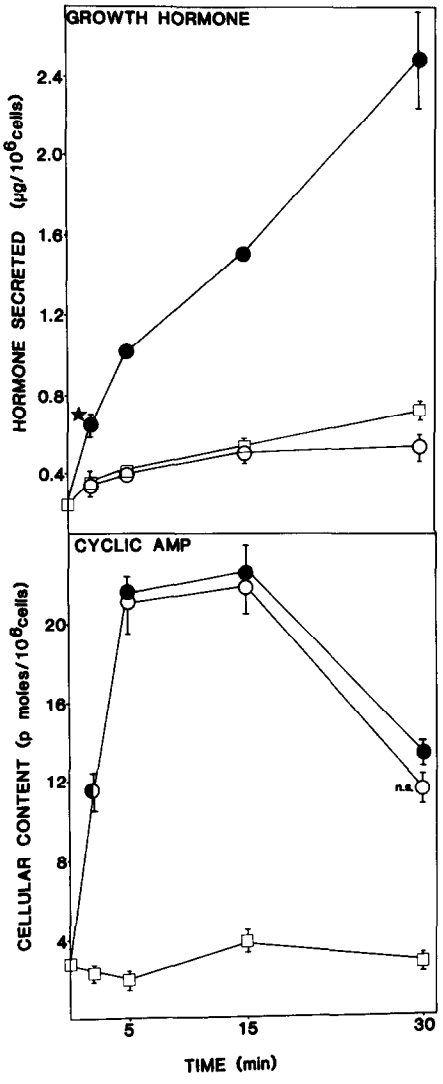

Fig.1. Time course of the effect of GRF on GH secretion in the absence or presence of SRIF (top) and cellular cyclic AMP levels (bottom). Sheep pituitary cells were cultured for 3 days and then treated with 1 nM GRF (●), 1 nM GRF plus 0.5 μM SRIF (○) or no additions (□). For each time point the values for hormones released and cyclic AMP content represent the mean values ± SE per 10^6 cells, obtained from 4 replicate dishes. The appropriate control and experimental values were compared for statistically significant differences by Student's *t*-test. Probability values: ★, $p < 0.001$; ns, not significant.

levels 5-fold within 2 min (fig.1). A maximal 10-fold elevation was apparent by 5 min (fig.1), and after 15 min the elevated cyclic AMP levels declined somewhat. SRIF had no significant effect on the time course.

3.3. *Dose dependence of the effect of SRIF on the GRF-stimulated GH secretion and cellular cyclic AMP levels in sheep pituitary cells*

The effects of various SRIF concentrations on GRF (0.1 nM)-stimulated GH secretion and cellular cyclic AMP levels after 30 min are shown in fig.2. GH secretion (open circles) was stimulated 2-fold, and SRIF inhibited this effect of GRF in a dose-dependent manner. Inhibition to below basal level was produced by 0.1 μM SRIF. On the other hand, over the dose range tested, SRIF had no significant effect on the 4-fold elevated cyclic AMP levels (closed circles) (fig.2). At higher SRIF concentrations (0.5 μM) we have occasionally noticed a (150–200%) stimulation of cellular cyclic AMP levels above that seen with GRF alone.

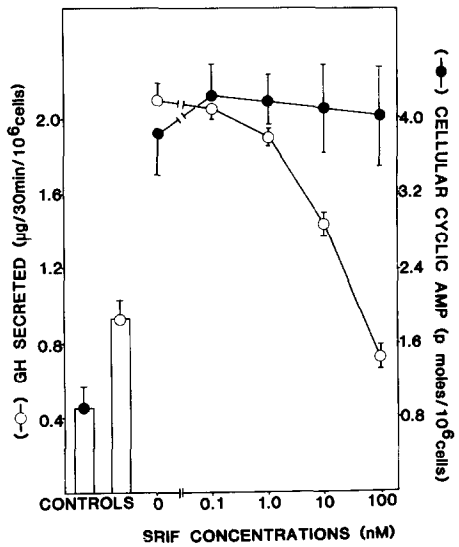

Fig.2. Dose-response relationships for the action of SRIF on GRF-stimulated GH secretion (○) and cyclic AMP content (●) in cultured sheep pituitary cells. For all observations except the controls 0.1 nM GRF was present, with the concentration of SRIF shown. For each treatment the values for GH released and cyclic AMP content represent the mean value ± SE per 10^6 cells, from 4 replicate dishes.

3.4. *Dose dependence of the effect of SRIF on GRF-stimulated GH secretion and cellular cyclic AMP levels in rat pituitary cells*

The results with rat pituitary cells are shown in fig.3. GH secretion was only stimulated 70% by 1 nM GRF after 30 min (open circles), though cellular cyclic AMP levels were elevated 32-fold (closed circles). An inhibition of the effects of GRF on both GH secretion and cyclic AMP levels was observed with SRIF, the dose-dependence curves apparently being parallel. A maximally effective dose of SRIF (1 nM) inhibited GH secretion to almost half the basal secretion value, whereas cellular cyclic AMP levels still remained 8-fold higher than the basal content.

4. DISCUSSION

Our data clearly show that SRIF does not inhibit the rapid GRF stimulation of cyclic AMP concentrations in sheep pituitary cells, although the peptide produces a rapid and potent inhibition of GRF-stimulated GH secretion. This indicates that the major site of action of SRIF in regulating GH secretion in these cells probably lies beyond cyclic AMP production. These findings accord with our earlier work with GRF [13], and with isobutylmethylxanthine and SRIF [18] using sheep pituitary cells, and also with the work of authors in [7] using bovine pituitary cells. They do not agree with previous studies using rat pituitary cells, which suggested that SRIF could inhibit the stimulation of adenylate cyclase caused by GRF [11,19,20]. Our results, presented here, clarify this situation. SRIF does indeed lower GRF-stimulated cyclic AMP levels in rat pituitary cells and an important species difference exists between rat and sheep cultured pituitary cells.

In these studies, which have used heterogeneous cell populations, it must be recognised that the non-somatotroph cell populations could be masking changes in cellular cyclic AMP levels in the sheep somatotrophs. However, this seems unlikely because although the magnitude of the difference observed between rat and sheep anterior pituitary cell preparations was very marked, somatotrophs constitute a similar and large (30–40%) proportion of the cells present. Further, since effects of GRF appear to be highly specific for GH secretion [9], changes in cyclic AMP levels induced by this factor should represent primarily changes occurring in the somatotroph.

Our results are consistent with the hypothesis that SRIF exerts its inhibitory effects on at least two sites in the rat pituitary cells [11,20]; one of these is at adenylate cyclase whilst the other is as yet undefined but is independent of cyclic AMP concentrations. In sheep cells the second site would appear to predominate. Modulation of cellular processes controlling Ca^{2+}-dependent stimulus-secretion coupling may be an alternative site for inhibitory regulation by SRIF [11]. Rat somatotrophs may display functional heterogeneity [21,22] and inhibition of adenylate cyclase by SRIF may operate predominantly in one subpopulation of the cells.

Authors in [19], using rat pituitary cells, showed that pertussis toxin (which inactivates the N_i inhibitory protein of adenylate cyclase by ADP ribosylation [23]) could block the inhibition by SRIF of both GRF-induced GH release and

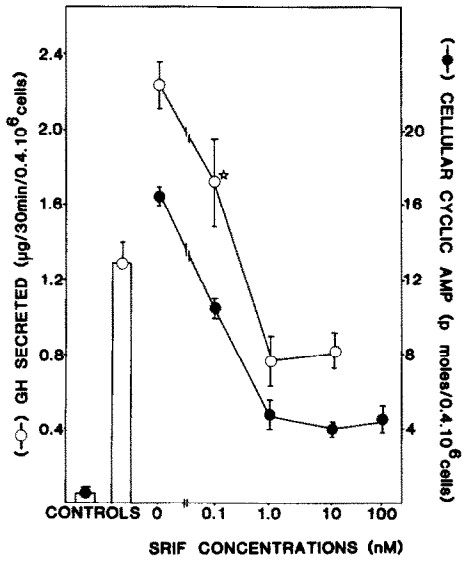

Fig.3. Dose-response relationships for SRIF acting on GRF-stimulated GH secretion (○) and cyclic AMP content (●) in cultured rat pituitary cells. For all observations except the controls 1.0 nM GRF was present, with the concentration of SRIF shown. For each treatment the values for GH released and cyclic AMP content represent the mean value ± SE per 0.4×10^6 cells, from 4 replicate dishes. Probability value: ☆, $p < 0.01$.

cellular cyclic AMP accumulation, which suggests that the inhibition of adenylate cyclase by SRIF is associated with its effects on GH secretion in the rat. However, our results and those in [11,20] show that SRIF-attenuated cyclic AMP concentrations in GRF-treated rat cells were still approx. 8-fold greater than the basal values, suggesting that inhibition of GRF-stimulated adenylate cyclase by SRIF may not entirely explain its effects on GH secretion, even in the rat.

It is noteworthy that the inhibitory potency of SRIF on GH secretion measured in the rat pituitary cell cultures (fig.3) was much greater than that observed in experiments with the sheep pituitary cells (fig.2). It is possible that in the rat cells the inhibition by SRIF of adenylate cyclase may potentiate its effects on other mechanisms involved in the inhibitory control of secretion.

In conclusion, the results presented here suggest that the lowering of cyclic AMP levels plays little role in the inhibition by SRIF of GRF-stimulated secretion of sheep GH. Lowering of cyclic AMP levels may play such a role in the rat, but its importance is not yet established.

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REFERENCES

[1] Schofield, J.G. (1967) Biochem. J. 103, 331–341.
[2] Wilber, J.F., Peake, G.T., Mariz, I., Utiger, R.F. and Daughaday, W.H. (1968) Clin. Res. 16, 277–280.
[3] Labrie, F., Béraud, G., Gauthier, M. and Lemay, A. (1971) J. Biol. Chem. 246, 1902–1908.
[4] Borgeat, P., Labrie, F., Drouin, J., Bélanger, A., Immer, H., Sestanj, K., Nelson, V., Gotz, M., Schally, A.V., Coy, D.H. and Coy, E.J. (1974) Biochem. Biophys. Res. Commun. 56, 1052–1059.
[5] Belanger, A., Labrie, F., Borgeat, P., Savary, M., Cote, J., Drouin, J., Schally, A.V., Coy, D.H., Coy, E.J., Immer, H., Sestanj, K., Nelson, V. and Gotz, M. (1974) Mol. Cell Endocrinol. 1, 329–339.
[6] Kaneko, T., Oka, H., Saito, S., Munemura, M., Masa, K., Oda, T., Yanaihara, N. and Yanaihara, C. (1973) Endocrinol. Jap. 20, 535–538.
[7] Bicknell, R.J. and Schofield, J.G. (1981) Mol. Cell Endocrinol. 22, 85–94.
[8] Sheppard, M.S., Spence, J.W. and Kraicer, J. (1979) Endocrinology 105, 261–268.
[9] Guillemin, R., Brazeau, P., Böhlen, P., Esch, F., Ling, N. and Wehrenberg, W.B. (1982) Science 218, 585–587.
[10] Rivier, J., Spiess, J., Thorner, M. and Vale, W. (1982) Nature 300, 276–278.
[11] Bilezikjian, L.M. and Vale, W.W. (1983) Endocrinology 113, 1726–1731.
[12] Michel, D., Lefèvre, G. and Labrie, F. (1983) Mol. Cell Endocrinol. 33, 255–264.
[13] Law, G.J., Ray, K.P. and Wallis, M. (1984) FEBS Lett. 166, 189–193.
[14] Labrie, F., Gagne, B. and Lefèvre, G. (1983) Life Sci. 33, 2229–2233.
[15] Ray, K.P. and Wallis, M. (1982) Mol. Cell Endocrinol. 27, 139–155.
[16] Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857.
[17] Brooker, G., Harper, J.F., Terasaki, W.L. and Moylan, R.P. (1979) Adv. Cyclic Nucleotide Res. 10, 1–33.
[18] Law, G.J., Ray, K.P. and Wallis, M. (1983) Abstracts, 2nd Joint Meeting of British Endocrine Societies, p.53.
[19] Cronin, M.J., Rogol, A.D., Myers, G.A. and Hewlett, E.L. (1983) Endocrinology 113, 209–215.
[20] Harwood, J.P., Grewe, C. and Aguilera, G. (1984) Mol. Cell Endocrinol. 37, 277–284.
[21] Snyder, G., Hymer, W.C. and Snyder, J. (1977) Endocrinology 101, 788–799.
[22] Hall, M., Howell, S.L., Schulster, D. and Wallis, M. (1982) J. Endocrinol. 94, 257–266.
[23] Hildebrandt, J.D., Sekura, R.D., Codina, J., Iyengar, R., Manclark, C.R. and Birnbaumer, L. (1983) Nature 302, 706–709.