Growth hormone-releasing factor regulates growth hormone mRNA in primary cultures of rat pituitary cells

(prolactin mRNA/cytoplasmic dot hybridization/secretion)

GREGORY G. GICK*, FÜSÜN N. ZEYİN†, PAUL BRAZEAU†, NICHOLAS C. LING†, FREDERICK S. ESCH†, AND CARTER BANCROFT*

*Memorial Sloan-Kettering Cancer Center and Department of Molecular Biology and Virology, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, NY 10021; and †Laboratories for Neuroendocrinology, The Salk Institute for Biological Studies, La Jolla, CA 92037

Communicated by Roger Guillemin, November 21, 1983

ABSTRACT A peptide with high intrinsic activity for specifically stimulating the secretion of immunoreactive growth hormone (GH; somatotropin) has been characterized and reproduced by total synthesis. This peptide, human pancreatic growth hormone-releasing factor, 44-amino-acid form (hpGRF1-44-NH₂), was isolated from a tumor localized in the pancreas of a patient with acromegaly. We report here the effect of this growth hormone-releasing factor (GRF) on GH release and the GH mRNA levels in monolayer cultures of rat pituitary. The cytoplasmic dot hybridization technique was used to examine the effect of GRF on GH mRNA levels. Incubation of rat pituitary cultures with GRF for 72 hr resulted in a 2- to 2.5-fold increase in GH mRNA levels, and the maximal levels of stimulation were achieved at GRF concentrations as low as 1 fM. GRF did not stimulate prolactin release, nor did it affect specific prolactin mRNA levels in the pituitary cultures. We conclude that GRF is a potent and specific GH secretagogue that also affects specifically GH mRNA levels in normal pituitary cells.

The hypothalamus fulfills a dual function in the regulation of growth hormone (GH; somatotropin) release. An inhibitory factor, somatostatin, has been characterized since 1972, whereas the stimulatory factor [growth hormone-releasing factor (GRF) or somatotropin] has eluded investigators until recently. In order to circumvent the problem of low abundance of GRF in the hypothalamus, extrahypothalamic sources of GRF production were sought. A solid tumor of the pancreas obtained at surgery from a patient who had produced the clinical syndrome of acromegaly was used to isolate and characterize the primary structure of three peptides possessing high intrinsic GH-releasing activity called hpGRF-40, hpGRF-44, and hpGRF-37, in which hp designates human pancreatic (1). Based upon their common NH₂ terminal sequence and lower specific activities *in vitro*, hpGRF-40 and -37 are most probably proteolytic degradation products of hpGRF-44. Most recently the isolation and structural characterization of bovine (2), porcine (3), and murine (4) hypothalamic GRF have been reported. Both bovine and porcine GRF have 44 amino acid residues and share extensive structural homology with the 44-amino-acid form of hpGRF designated hpGRF1-44 NH₂.

The experiments reported here were conducted with the synthetic replicate of hpGRF1-44-NH₂ (henceforth referred to as GRF or somatotropin). GRF already has been shown to stimulate GH-release *in vivo* (5) and *in vitro* (6) without affecting release of other pituitary hormones. We report here that GRF also stimulates GH mRNA sequences in rat pituitary cells. A portion of these findings has been presented in preliminary form (7).

MATERIALS AND METHODS

Rat Pituitary Cell Culture. Anterior pituitary glands were obtained from male Sprague-Dawley rats, 120-180 g. The method of dissociation and plating of cells are described in detail elsewhere (6).

RIA. RIAs for rat GH were performed with Sinha's anti-murine GH antiserum (8). Prolactin (PRL) was measured with the anti-PRL antiserum provided by the National Pituitary Agency (A. Parlow, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases).

Quantitation of GH and PRL mRNA Sequences. Relative levels of GH and PRL mRNA were measured by cytoplasmic dot hybridization as described (9, 10). Two dilutions of each cytoplasmic sample were dotted onto each of three sheets of nitrocellulose, each of which was then hybridized with a ³²P-labeled hybrid plasmid containing either GH cDNA (11), PRL cDNA (10), or 18S ribosomal DNA (12). The results with the latter probe were used to correct for variations in total cytoplasm applied to each dot.

Materials. Synthetic GRF was prepared by solid-phase synthesis (1). Culture media (F10 and F12) are from GIBCO, and fetal calf serum is from Sterile Systems (Logan, UT).

RESULTS AND DISCUSSION

Incubation of rat pituitary cells with 0.2 nM GRF resulted in a 2.4-fold induction of GH mRNA levels as compared to the untreated controls (Fig. 1). The time course of response of rat pituitary cells to GRF is shown in Fig. 2. Incubation of cells with 1.0 nM GRF yielded a 2.4-fold stimulation of GH secretion by day 1 (Fig. 2 *Upper Left*), and the response to GRF did not fluctuate significantly throughout the 4-day treatment period. In contrast, PRL secretion was unaffected by GRF (Fig. 2 *Lower Left*). To date, *in vitro* studies have focused primarily on the acute (i.e., 3-4 hr) response of pituitary cells to GRF (6). Results shown in Fig. 2 indicate that the high specificity of GRF for stimulation of GH secretion is also maintained during prolonged incubation (up to 4 days) of rat pituitary cells with GRF. Incubation of rat pituitary cells with 1.0 nM GRF for 1-4 days yielded a significant 2-fold stimulation of cytoplasmic GH mRNA sequences (Fig. 2 *Upper Right*), whereas it did not have a detectable effect on cytoplasmic PRL mRNA sequences (Fig. 2 *Lower Right*). This finding shows the specificity of GRF upon pituitary GH mRNA levels. The significance of the apparent 2-day lag preceding the stimulation of GH mRNA is presently not clear because it is not consistently observed (e.g., Fig. 1).

Incubation of pituitary cells for 3 days with concentrations of GRF ranging from 1 fM to 1 nM resulted in a 2-fold increase in GH release (Fig. 3 *Upper Left*) without changing

FIG. 1. Regulation of GH mRNA levels by GRF. Dispersed monolayer cultures of male rat pituitary cells were incubated in serum-free medium (see *Materials and Methods*) for 24 hr in the presence or absence of 0.2 nM GRF. Each value represents the mean $\pm$ SEM; $N = 6$ for -GRF and $N = 5$ for +GRF.

PRL secretion (Fig. 3 Lower Left). A 2.5-fold stimulation of GH mRNA levels, as compared to levels in control cultures, was observed at the lowest concentration of GRF used (1.0 fM). GH mRNA sequences were stimulated 2- to 2.5-fold in the concentration range tested (Fig. 3 Upper Right), whereas PRL mRNA sequences were not significantly altered by any one of the concentrations of GRF that were tested (Fig. 3 Lower Right). Thus, maximal stimulation of GH release and GH mRNA sequences occurred after the 3-day incubation with 1 fM of GRF. The correlation in time and concentration between GRF-induced GH release and specific GH mRNA levels may be indicative of common pathways regulating

FIG. 2. Kinetics of the effect of GRF on GH and PRL secretion and mRNA levels. Rat pituitary cells were incubated in serum-free medium in the presence of 1.0 nM GRF for up to 4 days. At the indicated times, GH secretion (Upper Left), PRL secretion (Lower Left), GH mRNA (Upper Right), and PRL mRNA levels (Lower Right) were determined. In this experiment pituitary cells at each time point had been in culture for the same length of time (a total of 8 days) and, thus, the day 0 time point represents the minus GRF control for the subsequent day 1 through day 4 experimental points. Each value represents the mean $\pm$ SEM; when no bar appears, SEM is no greater than the height of the symbol. Day 0 data in the GH and PRL secretion study represent the value of 16 cultures; $N = 8$ for the day 1 through day 4 values. The day 0 value in the GH and PRL mRNA analysis is derived from 10–11 replicate cultures, and experimental points represent the mean $\pm$ SEM from 4–6 cultures.

FIG. 3. Effect of various concentrations of GRF on GH and PRL secretion and mRNA levels. Primary cultures of rat pituitary cells were incubated as in Fig. 1 for 3 days in the presence of various concentrations of GRF, and GH secretion (Upper Left), PRL secretion (Lower Left), GH mRNA (Upper Right), and PRL mRNA sequences (Lower Right) were assayed. The zero GRF concentration points in the secretion analysis represent the value of 16 replicate cultures; $N = 8$ for the experimental points. Six replicate cultures were analyzed at the zero GRF concentration point in the GH and PRL mRNA determinations; $N = 5$ for all experimental points.

GH secretion and GH mRNA levels in pituitary cells.

We conclude that GRF has a highly specific and potent action on GH mRNA sequence levels in rat pituitary cells in monolayer culture. Our findings indicate that this system provides an excellent model for further characterizing the effects of GRF on the expression of the GH gene into RNA and on translation and post-translational modification of its protein product. The normal cells thus afford a sensitive and reproducible means of studying hormonal regulation of GH and the effect of other physiological regulators upon the action of GRF.

Note Added in Proof. After submission of this manuscript, a report appeared describing transcriptional effects of GRF very similar in magnitude and specificity to those described here (13). Taken together, these two reports suggest that there is little post-transcriptional regulation by GRF of GH gene expression.

This work was supported by National Institutes of Health Grants AM-24442, CA-08748, HD-09690, and AM-18811. We thank Drs. R. Maurer (University of Iowa) and R. B. Guntaka (Columbia University) for supplying plasmids containing PRL cDNA (pPrl-1) and 18S ribosomal DNA (pXC-1), respectively. We thank Marilyn McLaughlin and Ginny Page for expert technical assistance and the secretarial staff of the Laboratories for Neuroendocrinology at the Salk Institute for typing the manuscript.

- Guillemin, R., Brazeau, P., Böhlen, P., Esch, F., Ling, N. & Wehrenberg, W. (1982) *Science* **218**, 585–587.
- Esch, E., Böhlen, P., Ling, N., Brazeau, P. & Guillemin, R. (1983) *Biochem. Biophys. Res. Commun.*, in press.
- Böhlen, P., Esch, F., Brazeau, P., Ling, N. & Guillemin, R. (1983) *Biochem. Biophys. Res. Commun.* **116**, 726–734.
- Spiess, J., Rivier, J. & Vale, W. (1983) *Nature (London)* **303**, 532–535.
- Wehrenberg, W. B., Ling, N., Brazeau, P., Esch, F., Böhlen, P., Baird, A., Ying, S.-Y. & Guillemin, R. (1982) *Biochem. Biophys. Res. Commun.* **109**, 382–387.
- Brazeau, P., Ling, N., Böhlen, P., Esch, F., Ying, S.-Y. & Guillemin, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7909–7913.
- Gick, G. G., Zeytinoglu, F. N., Esch, F. S. & Bancroft, F. C. (1983) in *Program of the 65th Annual Meeting of the Endocrine Society*, San Antonio, TX, Abstr. 13.

8. Sinha, Y. N., Selby, F. W., Lewis, J. G. & Vanderlaan, W. P. (1972) *Endocrinology* **91**, 784–792.
9. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257**, 8569–8572.
10. White, B. A. & Bancroft, F. C. (1983) *J. Biol. Chem.* **258**, 4618–4622.
11. Dobner, P. R., Kawasaki, E. S., Yu, L.-Y. & Bancroft, F. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2230–2234.
12. Katz, R. A., Erlanger, B. F. & Guntaka, R. V. (1983) *Biochim. Biophys. Acta* **739**, 258–264.
13. Barinaga, M., Yamamoto, G., Rivier, C., Vale, W., Evans, R. & Rosenfeld, M. G. (1983) *Nature (London)* **306**, 84–85.