Vol. 124, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1984 Pages 217-222

ADMINISTRATION OF HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR (GRF) ANALOGS ENHANCES RESPONSIVENESS OF CULTURED RAT PITUITARY CELLS TO GRF

MARK L. HEIMAN, WILLIAM A. MURPHY, MARY V. NEKOLA, VALENTINE A. LANCE AND DAVID H. COY.

DEPARTMENT OF MEDICINE, TULANE UNIVERSITY SCHOOL OF MEDICINE, NEW ORLEANS, LA 70112

Received August 29, 1984

The aim of this study was to investigate whether anterior pituitary responsiveness to human pancreatic growth hormone-releasing factor containing 29 amino acids (GRF-29) can be modulated by GRF-29 itself. Male rats were injected (sc) daily for 3 days with 50 ug of GRF-29, or were treated twice daily for 14 days with 5 ug of [D-Ala-2]-GRF-29 (a potent GRF agonist). Control animals were injected with saline. After the last injection, pituitaries were removed, dispersed, cultured for 96 h and then challenged with either GRF-29 or [D-Trp-6]-LHRH (a LHRH agonist). Cultured cells from analog-treated rats were more responsive to GRF-29 stimulation than were cells obtained from controls. In contrast, neither treatment altered the response to [D-Trp-6]-LHRH. These studies indicate that periodic administration of GRF analogs can increase hypophyseal GRF responsiveness. Such control may be an important component in the physiological regulation of GH secretion and has important implications for potential therapeutic uses of GRF analogs.

Physiological regulation of anterior pituitary hormone secretion is dependent, in part, on the responsiveness of the target cell to its respective hypothalamic hormone(s). For example, pituitary sensitivity to LHRH (1,2) changes immediately prior to the spontaneous preovulatory LH surge. Modulation of such responsiveness can be accomplished by the hypothalamic hormone itself, by other circulating hormones, or by both. Indeed, estradiol both inhibits and augments pituitary responsiveness to LHRH (3,4) and LHRH enhances pituitary responsiveness to itself (5-7).

Regulation of GH secretion from the pituitary is mediated in part by a hypothalamic inhibitory hormone (somatostatin) and a hypothalamic releasing factor (GRF). Recently, two peptides, structurally characterized from human pancreatic tumors, were reported to be highly potent in stimulating GH

Abbreviations: LHRH, luteinizing hormone-releasing hormone; LH, luteinizing hormone; GH, growth hormone; GRF, growth hormone-releasing factor; GRF-29, GRF(1-29)NH_2.

0006-291X/84 $1.50

secretion (8,9). Moreover, pituitary sensitivity to synthetic GRF is increased by glucocorticoids (10,11) and triiodothyronine (10). The aim of the present study was to investigate whether an additional modulator of GRF responsiveness could be provided by GRF itself.

MATERIALS AND METHODS

Animals and Cell Culture

Adult male Sprague-Dawley rats, weighing 250-300 g and housed under controlled temperature and lighting conditions (lights on from 0500-1900 h) were used. Food and water were available ad libitum. Groups of 6-7 rats were injected (sc) daily for 3 days at 1500 h with either 50 ug of GRF-29 (a shortened N-terminal GRF analog that is as active as its parent-44 amino acid peptide (9)) or saline. Other rats were injected (sc) twice daily (0900 and 1500 h) for 14 days with either 5 ug of [D-Ala-2]-GRF-29 (a potent GRF agonist (12)) or saline. On the morning after the last injection, rats were killed by decapitation and anterior pituitary cells were dispersed and cultured for 96 h by a previously described method (13, 14). Cells were then challenged with either GRF-29 (doses ranging from 0.1 fM to 60 fM) or [D-Trp-6]-LHRH (ranging from 0.006 nM to 2 nM) for 3 h at 37 C. Each dose was administered to the cells in triplicate.

Synthetic Peptides

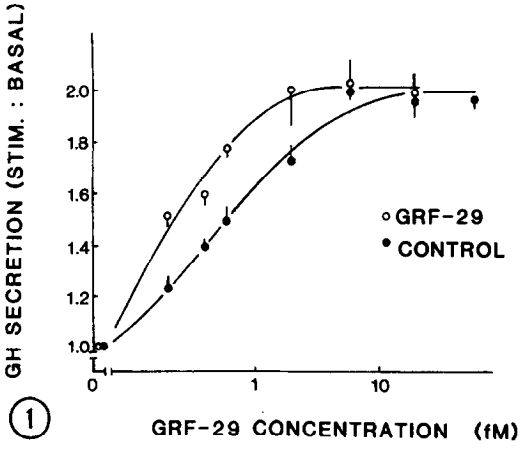
GRF-29 (GRF(1-29)-NH_2), [D-Ala-2]-GRF-29, and [D-Trp-6]-LHRH were synthesized by solid-phase methodology and purified by multiple preparative and high performance reverse-phase liquid chromatographic steps as previously described (15).

Hormone Assay and Data Analysis

Measurement of rat GH and LH in the media were performed in duplicate by a standard double antibody RIA using reagents kindly supplied by the National Pituitary Agency and the NIADDK. Differences in GRF-29 and [D-Trp-6]-LHRH responsiveness of the cells were analyzed by comparing the dose-response data obtained from the treated group with that observed from its control using the computer program ALLFIT described by DeLean et al.(16).

RESULTS

Pituitary cells plated from the rats treated with GRF-29 were more responsive to GRF-29 stimulation than were cells cultured from their saline injected counterparts (Fig. 1). The median effective GRF-29 concentration (EC-50) calculated for the treated groups was significantly ($p < 0.05$) smaller than that computed for their controls in each of 3 different experiments. A significant ($p < 0.05$) increase in pituitary responsiveness to GRF-29 was also evident in cells cultured from animals treated with [D-Ala-2]-GRF-29 (Fig. 2). This effect was observed in each of two separate experiments. Slopes of each dose-response curve, as well as the stimulated to basal GH ratio (estimated



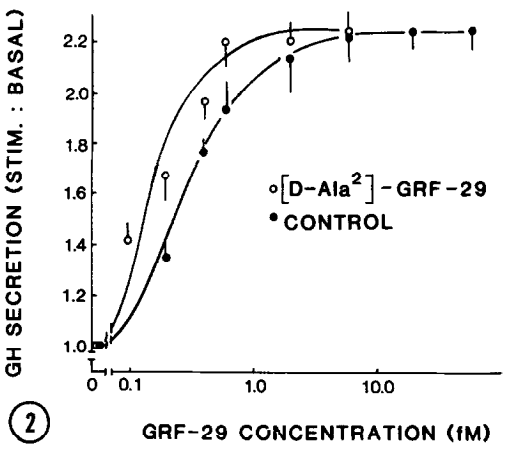
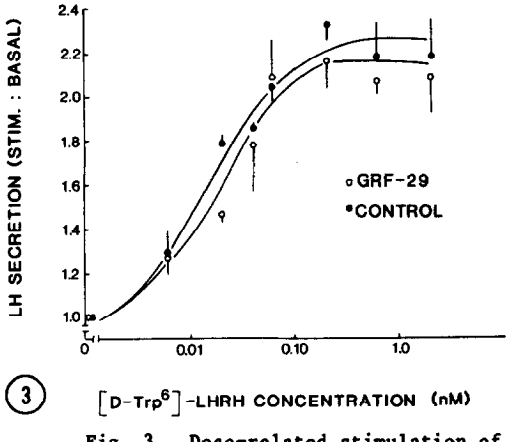


Fig. 1. Dose-related stimulation of GRF-29 mediated GH secretion (stimulated to basal ratio). Cultured cells were prepared from rats injected daily with either GRF-29 (o) or saline (•) for 3 days, as described in Materials and Methods. Each point is the mean ± SE of triplicate incubations of 200,000 cells/well. The data are from 1 of 3 similar experiments.

Fig. 2. Dose-related stimulation of GRF-29 mediated GH secretion (stimulated to basal ratio). Cells were prepared from rats after 2 weeks of treatment with either [D-Ala-2]-GRF-29 (o) or saline (•), as described in Materials and Methods. The data are from 1 of 2 similar experiments.

for an "infinite" GRF-29 dose), were unaffected by either treatment. In contrast, GRF analog administration did not affect [D-Trp-6]-LHRH pituitary responsiveness (Figs. 3 and 4).



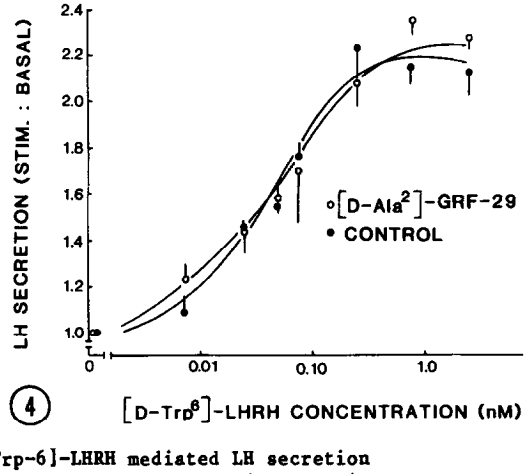


Fig. 3. Dose-related stimulation of [D-Trp-6]-LHRH mediated LH secretion (stimulated to basal ratio). Cells were prepared from rats injected daily with either GRF-29 (o) or saline (•) for 3 days as described in Materials and Methods. The data are from 1 of 3 similar experiments.

Fig. 4. Dose-related stimulation of [D-Trp-6]-LHRH mediated LH secretion (stimulated to basal ratio) in cultured cells. Cells were prepared from rats after 2 weeks of treatment with either [D-Ala-2]-GRF-29 (o) or saline (•), as described in Materials and Methods. The data are from 1 of 2 similar experiments.

DISCUSSION

Our results demonstrate that daily administration of GRF-29 for 3 days can enhance pituitary responsiveness to GRF-29. A similar increase in sensitivity was also observed after the longer treatment regimen with [D-Ala-2]-GRF-29. These analog mediated changes in pituitary sensitivity were not generalized for all cell types since [D-Trp-6]-LHRH responsiveness was unaltered.

We think these observations reflect the endocrine status of the animal at the time the pituitary cells were isolated. That pituitary cells isolated from animals in different physiological states retain, in short-term primary culture, their previous responsiveness to hypothalamic hormones has been previously documented (13, 17-19). Further, manipulation of the endocrine system *in situ* can be observed in cultured cells obtained from such treated animals. Indeed, castration of either male or female rats more than doubled the LHRH mediated LH and FSH release from pituitary cells cultured 7 days after gonadectomy (20). Moreover, the culture medium used for each treatment group was identical to that employed for control cells except for the inclusion of rat serum which was obtained from the respective pituitary donor groups. However, culturing cells derived from control animals in medium that was supplemented with serum from either GRF-29 or [D-Ala-2]-GRF-29 treated rats did not affect cell responsiveness to GRF-29 (not shown). Therefore, it is unlikely that the data resulted from changes induced by the addition of sera from donor rats.

The responsiveness of pituitary cells to their hypothalamic hormones depends, in part, on the quantity of specific receptors available for binding. That the number of binding sites can alter the pituitary responses to its hypothalamic hormone has been demonstrated directly for TRH (21) and somatostatin (22). Additional evidence has been derived indirectly by correlation. The enhanced LHRH responsiveness of pituitary cells obtained from castrated rats and placed in culture (20) reflects the higher number of LHRH receptors after gonadectomy (23,24). Additionally, preincubation of

primary pituitary cultures with estradiol blunts the dopaminergic inhibition of PRL secretion (25), an effect that may be mediated by an estradiol-induced decrease in pituitary dopamine receptors (26). Thus, the increased GRF-29 cellular responsiveness observed in the present study could be mediated, in part, by an increase in the number of GRF receptors.

Pathophysiological states of ectopic GRF secretion from bronchial and pancreatic tumors are reported to be associated with chronically elevated GH levels and the clinical manifestations of acromegaly (27-30). Such clinical findings probably would not have been as apparent if the pituitaries of these patients were desensitized to the ectopically secreted GRF. Since synthetic GRF analogs are potent stimulators of GH release in the rat (8,9,12), it follows that administration of synthetic GRF analogs to rats could either enhance or not affect pituitary responsiveness to GRF. Indeed, our data suggest that such treatment increases GRF responsiveness, even following injections with a potent GRF agonist for 2 weeks. Such a long-term treatment regimen with a potent LHRH agonist desensitizes the pituitary to LHRH (31). The increase in hypophyseal sensitivity to GRF-29 may be attributed directly to the GRF analog treatments themselves. However, we cannot rule out the possible involvement of other hormones, secondary or tertiary to GRF stimulation, such as GH and the somatomedins. Recently, Wehrenberg et al. (32) and Dieguez et al. (33) reported that the rat hypophysis could be desensitized to GRF by constant exposure to GRF for 24 h. The discrepancy between these data and ours may be due to differences in GRF treatment regimen (intermittent vs. constant).

In conclusion, these data indicate that intermittent administration of GRF analogs increases pituitary responsiveness to GRF-29. Since [D-Trp-6]-LHRH sensitivity was unchanged after either short- or long-term treatment, this enhanced responsiveness to GRF-29 was not generalized for all cell types. Such a change in pituitary responsiveness may be an important component in the physiological regulation of GH, and could be of considerable value in the potential therapeutic uses of GRF agonists.

ACKNOWLEDGEMENTS

We thank Karen Brown, Vienna Mackey, and Lisa Mutty for their expert technical participation. We also acknowledge the administrative assistance of Anita Miller. The research was supported, in part, by NIH grant AM 30167 and The Mellon Foundation.

REFERENCES

1. Aiyer, M.S., Fink, G., and Greig, F. (1974). J. Endocrinol. 60,47-64.
2. Gordon, J.H. and Reichlin, S. (1974). Endocrinology 94,974-978.
3. Libertun, C., Orias, R., and McCann, S.M. (1974). Endocrinology 94,1094-1100.
4. Fink, G. (1979). Ann. Rev. Physiol. 41,571-585.
5. Aiyer, M.S., Chiappa, S.A., and Fink, G. (1974). J. Endocrinol. 62,573-588.
6. Castro-Vazquez, A., and McCann, S.M. (1975). Endocrinology 97,13-19.
7. Waring, D.W., and Turgeon, J.L. (1983). Am. J. Physiol. 244,C410-C418.
8. Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N., and Wehrenberg, W. (1982). Science 218,585-587.
9. Rivier, J., Spiess, J., Thorner, M., and Vale, W. (1982). Nature 300,276-278.
10. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J., and Rivier, J. (1983). Endocrinology 112,1553-1555.
11. Wehrenberg, W.B., Baird, A., and Ling, N. (1983). Science 221,556-558.
12. Lance, V.A., Murphy, W.A., Sueiras-Diaz, J., and Coy, D.H. (1984). Biochem. Biophys. Res. Commun. 119,265-272.
13. Hoefer, M.T., Heiman, M.L., and Ben-Jonathan, N. (1984). Mol. Cell. Endocrinol. 35,229-235.
14. Heiman, M.L., Nekola, M.V., Murphy, W.A., Lance, V.A., and Coy, D.H. (1984). Endocrinology (In press).
15. Coy, D.H., Murphy, W.A., Sueiras-Diaz, J.J., Coy, E.J., and Lance, V.A. (1984). J. Med. Chem. (In Press).
16. DeLean, A., Munson, P.J., and Rodbard, D. (1978). Am. J. Physiol. 235,E97-E102.
17. Denef, C., Hautekeete, E., and Dewals, R. (1978). Endocrinology 103,736-747.
18. Nakano, H., Fawcett, C.P., and McCann, S.M. (1976). Endocrinology 98,278-288
19. Tang, L.K., and Tang, F.Y. (1979). Am. J. Physiol. 236,E216-E221.
20. O'Conner, J.L., Allen, M.B., and Mahesh, V.B. (1980). Endocrinology 106,1706-1714.
21. Perrone, M.H., and Hinkle, P.M. (1978). J. Biol. Chem. 253,5168-5173.
22. Schonbrunn, A., and Tashjian, A.H. (1978). J. Biol. Chem. 253,6473-6483.
23. Clayton, R.N., and Catt, K.Y. (1981). Endocrinology 108,887-895.
24. Frager, M.S., Pieper, D.R., Tonetta, S., Duncan, J.A., and Marshall, J.C. (1981). J. Clin. Invest. 67,615-623.
25. Raymond, V., Beaulieu, M., and Labrie, F. (1978). Science 200,1173-1175.
26. Heiman, M.L., and Ben-Jonathan, N. (1982). Endocrinology 111,1057-1060.
27. Zafar, M.S., Mellinger, R.C., Fine, G., Szabo, M., and Frohman, L.A. (1979). J. Clin. Endocrinol. Metab. 48,66-71.
28. Frohman, L.A., Szabo, M., Berelowitz, M., and Stachura, M.E. (1980). J. Clin. Invest. 65,43-54.
29. Leveston, S.A., McKeel, D.W., Buckley, P.J., Deschyver, K., Greider, M.H., Jaffe, B.M., Daughaday, W.H. (1981). J. Clin. Endocrinol. Metab. 53,682-689.
30. Thorner, M.O., Perryman, R.L., Cronin, M.J., Rogol, A.D., Draznin, M., Johanson, A., Vale, W., Horvath, E., and Kovacs, K. (1982). J. Clin. Invest. 70,965-977.
31. Sandow, J. (1982). Neuroendocrine Perspectives Vol. 1, (eds. Muller, E.E., and MacLeod, R.M.), pp. 339-395, Elsevier Biomedical Press, Amsterdam.
32. Wehrenberg, W.B., Brazeau, P., Ling, N., Textor, G., Guillemin, R. (1984). Endocrinology 114,1613-1616.
33. Dieguez, C., Foord, S.M., Shewring, G., Edwards, C.A., Heyburn, P.J., Peters, J.R., Hall, R., and Scanlon, M.F. (1984). Biochem. Biophys. Res. Commun. 121,111-117.