INHIBITION OF ADENOSINE 3',5'-MONOPHOSPHATE ACCUMULATION IN ANTERIOR PITUITARY GLAND IN VITRO BY GROWTH HORMONE-RELEASE INHIBITING HORMONE

Pierre Borgeat, Fernand Labrie, Jacques Drouin and Alain Bélanger

MRC Group in Molecular Endocrinology,
Centre Hospitalier de l'Université Laval,
Quebec, Canada G1V 4G2

Hans Immer, K. Sestanj, V. Nelson and Manfred Götz,

Ayerst Research Laboratories, Montreal,
Canada.

Andrew V. Schally, David H. Coy and Esther J. Coy,

Veterans Administration Hospital and Tulane
University School of Medicine, New Orleans,
Louisiana 70146

Received December 26,1973

SUMMARY. 1 x 10^{-7}M growth hormone-release inhibiting hormone (GH-RIH or somatostatin) leads to a 50% inhibition of adenosine 3',5'-monophosphate (cyclic AMP) accumulation in anterior pituitary tissue during the first 2 min of incubation. This lowering of cyclic AMP levels is accompanied by inhibition of the release of immunoreactive growth hormone and thyrotropin. GH-RIH has also a marked inhibitory effect on the theophylline- and prostaglandin E_2-induced accumulation of adenohypophyseal cyclic AMP.

Secretion of growth hormone by the anterior pituitary gland is regulated by the delicate interplay of stimulatory and inhibitory substances secreted by the hypothalamus (1-4). The neurohormone which stimulates GH release has been named GH-releasing hormone (GH-RH) (2) and has been purified from porcine, sheep and rat hypothalami (5-7). Recently, the tetradecapeptide H-Ala-Gly-

Abbreviations: GH, growth hormone; GH-RIH, GH-release inhibiting hormone; GH-RH, GH-releasing hormone; TSH, cyclic AMP adenosine 3',5'-monophosphate; KRBG, Krebs Ringer bicarbonate buffer containing 11 mM D-glucose.

Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH inhibiting the release of immunoreactive GH both *in vivo* and *in vitro* has been isolated from ovine hypothalami (8-10) and called somatostatin (8) or GH-RIH (11).

Using a purified fraction of porcine GH-RH, we have recently obtained convincing evidence for a role of cyclic AMP as mediator of the action of the neurohormone stimulating GH release in the anterior pituitary gland (12-15). It was thus felt of interest to investigate a possible action of GH-RIH, the neurohormone inhibiting GH release, on the adenohypophyseal adenylate cyclase system.

MATERIALS AND METHODS

Hemipituitaries from adult male Sprague-Dawley rats (225-250g) were prepared and incubated (8 per group) as described (14,16) and indicated in the Legends to Tables and Figures. Pituitary cyclic AMP was measured (16) using the receptor-binding assay of Gilman (17). Before assay, the cyclic nucleotide was purified on Dowex AG-1-X8 (formate form). The standard extract was applied onto the column in 0.1 N HCl and, after washing with 10 ml H_2O, cyclic AMP was eluted with 10 ml 2 N formic acid. Data of triplicate determinations are presented as mean ± S.D.

GH and TSH release was measured by double-antibody radioimmunoassay (18) using rat hormones (GH-I-2, GH-RP-1, TSH-I-1 and TSH-RP-1) and rabbit antisera (anti GH-S-2 and anti-TSH-S-1) generously supplied by the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program. Radioimmunoassay data were analysed with an IBM-360-APL computer essentially as described. GH-RIH (AY 24,910) was synthesized by classical fragment synthesis

(20); PGE_2 was a generous gift from Dr. John E. Pike, Upjohn Co., Kalamazoo.

RESULTS

Table 1 shows that 1 x 10^{-7}M GH-RIH leads to a 48% inhibition

Table 1 Time course of the effect of 1 x 10^{-7}M GH-RIH on the accumulation of cyclic AMP in rat hemipituitaries.

Minutes of incubation	pmoles cyclic AMP/ anterior pituitary		% of control
	Control	GH-RIH	
2	16 ± 1.5	8.3 ± 0.9	52
5	6.9 ± 0.9	4.4 ± 0.2	63
15	7.6 ± 0.6	6.0 ± 1.2	79
30	6.9 ± 1.2	6.1 ± 0.1	88
60	6.6 ± 0.1	5.8 ± 0.9	89
180	5.5 ± 1.0	5.5 ± 0.4	100

After a preliminary incubation of 1.5 hr in Krebs Ringer bicarbonate buffer containing 11 mM D-glucose (KRBG) (14, 16), hemipituitaries (8 per group) were incubated in the presence or absence of 1 x 10^{-7}M GH-RIH for the indicated time periods.

of adenohypophyseal cyclic AMP accumulation during the first 2 min of incubation. Inhibitions of 37 and 21% are then found after respectively 5 and 15 min of incubation with GH-RIH with a progressive return to basal levels at later time intervals.

Under similar experimental conditions, the release of immunoreactive growth hormone (GH) and thyrotropin (TSH) was inhibited to respectively 52 and 71% of the control rate after 2 min of incubation with GH-RIH (Table 2). An approximately 50% inhibition

Table 2 Time course of the effect of GH-RIH on the basal release of GH and TSH

Minutes of incubation	GH released (μg GH-RP-1/ml)			TSH released (μg TSH-RP-1/ml)		
	Control	GH-RIH	% control	Control	GH-RIH	% control
2	2.4 ± 0.4	1.3 ± 0.1	52	3.8 ± 0.5	2.7 ± 0.7	71
5	1.4 ± 0.1	0.6 ± 0.05	46	4.8 ± 0.4	1.8 ± 0.1	38
15	5.7 ± 1.3	2.7 ± 0.3	47	16.6 ± 2.2	7.1 ± 1.3	43
30	10.8 ± 1.0	4.4 ± 0.2	41	43.7 ± 5.0	33.5 ± 6.6	77
60	18.5 ± 1.3	13.9 ± 1.0	75	47.4 ± 7.0	37.8 ± 3.7	80
120	38.3 ± 3.1	25.1 ± 2.0	66	136.0 ± 29.6	170.5 ± 30.7	125
180	68.2 ± 2.3	52.2 ± 3.5	76	321.6 ± 36.7	229.7 ± 13.1	71

After a preliminary incubation of 2 hr at 37°, male rat hemipituitaries (3 per group) were incubated in the presence or absence of 1×10^{-7}M GH-RIH for the indicated time intervals. GH and TSH in the incubation medium were measured by radioimmunoassay. Data are presented as mean ± S.E.M.

was then measured up to 30 and 15 min respectively for GH and TSH with a progressive return toward basal levels at later time intervals. That this transient effect of GH-RIH is due to inactivation of the tetradecapeptide and not to a characteristic of the adenohypophyseal adenylate cyclase system is indicated by a constant inhibition of release of newly-synthetized GH up to 3 hr when the neurohormone is added every 30 min (21).

Since GH-RIH does not inhibit only the basal but also the N^6-monobutyryl cyclic AMP-, theophylline- and PGE_2-induced release of GH and TSH, it was of interest to investigate a possible effect of GH-RIH on the stimulation of adenohypophyseal cyclic AMP induced by theophylline, an inhibitor of cyclic nucleotide phospho-

Table 3 Effect of GH-RIH on the theophylline- and PGE_2-induced accumulation of cyclic AMP in rat anterior pituitary gland *in vitro*.

ADDITION	pmoles cyclic AMP/ anterior pituitary	% of control
None	7.0 ± 1.8	500
Theophylline	35 ± 3.1	
Theophylline	60 ± 0.3	43
Theophylline + GH-RIH	26 ± 1.9	
None	4.2 ± 1.0	1390
PGE_2	58 ± 2.8	
PGE_2	57 ± 4.1	77
PGE_2 + GH-RIH	44 ± 0.8	

After preincubation of paired hemipituitaries for 2.5 hrs, the incubation was performed for 60 min in the presence or absence of 10 mM theophylline, 2×10^{-6}M PGE_2 or 1×10^{-7}M GH-RIH.

diesterase, and by PGE_2, an activator of adenohypophyseal adenylate cyclase (14). As shown in Table 3, the 5-fold stimulation of pituitary cyclic AMP concentration induced by 10 mM theophylline is reduced to 43% after 1 hr of incubation in the presence of GH-RIH while the neurohormone leads to a 23% inhibition of the 14-fold increased cyclic AMP accumulation induced by 2×10^{-6}M PGE_2 under similar experimental conditions. When a higher concentration of GH-RIH is used (2×10^{-6} instead of 1×10^{-7}), the stimulation of adenohypophyseal cyclic AMP accumulation is reduced by 45 to 60% up to 3 hr of incubation (Table 4).

DISCUSSION

The present data show that GH-RIH leads to a rapid inhibition

of cyclic AMP accumulation in anterior pituitary gland *in vitro* (Table 1) and this lowering of adenohypophyseal cyclic AMP levels is accompanied by a marked inhibition of both GH and TSH release (Table 2).

Since GH- and TSH-secreting cells account for 50 to 70% of adenohypophyseal cells in adult male rats, the 50% inhibition of cyclic AMP accumulation in total pituitary tissue suggests an almost complete inhibition of cyclic AMP accumulation in the GH and TSH cells. In fact, although no information is yet available on the effect of GH-RIH on corticotrophs, no effect of the neurohormone has been found on luteinizing hormone and prolactin release (21).

Although the inhibitory effect of GH-RIH on basal and PGE_2-induced pituitary cyclic AMP accumulation could suggest an action of the tetradecapeptide at a step preceding cyclic AMP formation, the finding (Table 3) of a 57% inhibition of the cyclic AMP accumulation induced by 10 mM theophylline would indicate an action of GH-RIH at a step following cyclic AMP formation. Such possi-

Table 4 Time course of the effect of 2×10^{-6}M GH-RIH on the accumulation of cyclic AMP in rat hemipituitaries in the presence of PGE_2.

Minutes of incubation	pmoles cyclic AMP/ anterior pituitary		% of control
	Control	GH-RIH	
5	47 ± 4	27 ± 1	55
15	125 ± 9	44 ± 3	35
45	138 ± 7	68 ± 5	49
90	100 ± 5	40 ± 2	40
180	108 ± 9	41 ± 2	39

Incubation conditions were as in Table 1 except for the presence of 1×10^{-6}M PGE_2.

bility is strengthened by the marked inhibition by GH-RIH of both GH and TSH release induced by N^6-monobutyryl cyclic AMP (21).

REFERENCES

1. Deuben, R. and Meites, J. Endocrinology 74, 408-414 (1964).
2. Schally, A.V., Arimura, A., Bowers, C.Y., Kastin, A.J., Sawano, S. and Redding, T.W. Rec. Progr. Horm. Res. 24, 497-508 (1968).
3. McCann, S.M. and Porter, J.C. Physiol. Rev. 49, 240-284 (1969).
4. Krulich, L., Dhariwal, A.P.S. and McCann, S.M. Endocrinology 83, 783-790 (1968).
5. Schally, A.V., Sawano, S., Arimura, A., Barret, J.F., Wakabayashi, I. and Bowers, C.Y. Endocrinology 84, 1493-1506 (1969).
6. Wilber, J., Nagel, T. and White, W.F. Endocrinology 89, 1419-1424 (1971).
7. Malacara, J.M. and Reichlin, S. Fed. Proc. 30, 198 (Abstract) (1971).
8. Brazeau, P., Vale, W., Burgus, R., Ling, V., Butcher, M., Rivier, J. and Guillemin, R. Science 179, 77-79 (1973).
9. Vale, W., Brazeau, P., Grant, G., Nussey, A., Burgus, R., Rivier, J., Ling, N. and Guillemin, R. C.R. Acad. Sci. Paris Serie D 275, 2913-2916 (1972).
10. Rivier, R., Brazeau, P., Vale, W., Ling, N., Burgus, R., Gilin, C., Yardley, J. and Guillemin, R. C.R. Acad. Sci. Paris Serie D 276, 2737-2742 (1973).
11. Coy, D.H., Coy, E.J. and Schally, A.V. Biochem. Biophys. Res. Commun. 54, 1267-1273 (1973).
12. Borgeat, P., Labrie, F., Poirier, G., Chavancy, G. and Schally, A.V. Trans. Am. Phys. Ass. in press (1973).
13. Labrie, F., Pelletier, G., Lemay, A., Borgeat, P., Barden, N., Dupont, A., Savary, M., Coté, J. and Boucher, R. In Sixth Karolinska Symp. on Research Methods in Reprod. Endocrinol. (ed Diczfalusy, E.) 301-340.
14. Labrie, F., Gauthier, M., Pelletier, G., Borgeat, P., Lemay, A. and Gouge, J.J. Endocrinology 93, 903-914 (1973).
15. Borgeat, P., Poirier, G., Chavancy, G., Dupont, A. and Labrie, F. In Hypothalamic Hypophysiotropic Hormones: Clinical and Physiological studies (eds Gual C. and Rosenberg) Excerpta Medica, Amsterdam, 174-181.

16. Borgeat, P., Chavancy, G., Dupont, A., Labrie, F., Arimura, A. and Schally, A.V. Proc. Nat. Acad. Sci. USA 69, 2677-2681 (1972).

17. Gilman, A.G. Proc. Nat. Acad. Sci. USA 67, 305-312 (1972).

18. Birge, C.A., Peake, G.T., Mariz, I.K. and Daughaday, W.H. Endocrinology 81, 195-204 (1967).

19. Rodbard, D. In Principles of Competitive Protein-Binding Assays (eds Odell, W.D. and Daughaday, W.H.), 204-259. The J.B. Lippincott Co.

20. Immer, H., Sestanj, K., Nelson, V. and Gotz, M. (manuscript in preparation) (1974).

21. Labrie, F., Drouin, J., Borgeat, P., Savary, M., Coté, J., Schally, A.V., Coy, D.H., Coy, E.J., Immer, H., Sestanj, K., Nelson, V. and Gotz, M. (manuscript in preparation) (1974).