THYROTROPIN RELEASING HORMONE: DIRECT EVIDENCE FOR STIMULATION OF PROLACTIN PRODUCTION BY PITUITARY CELLS IN CULTURE

A. H. Tashjian, Jr., N. J. Barowsky and D. K. Jensen

Pharmacology Department, Harvard School of Dental Medicine and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115.

Received March 10, 1971

SUMMARY

Addition of thyrotropin releasing hormone (TRH) to the medium of 2 clonal strains of functional rat pituitary cells stimulated the production of prolactin and inhibited growth hormone production. There was no effect on cell growth. Stimulation of prolactin production by TRH was detected within 4 hr, it reached a maximum level (2-5 times control) at 24-48 hr and persisted for at least 20 days in the continued presence of TRH. Stimulation was observed with a concentration of TRH as low as 0.10 ng/ml.

INTRODUCTION

The hypothalamus produces a group of factors which play an important role in the release of many and in the inhibition of release of some of the hormones of the anterior pituitary gland (1,2). Effects of these substances on the biosynthesis of pituitary hormones remain uncertain (3). There is both physiological and chemical evidence that the hypothalamic factors may be highly specific for each of the pituitary hormones (1,2). Recently, the structure of thyrotropin releasing factor or hormone (TRH) has been shown to be the tripeptide, L-(pyro)Glu-L-His-L-Pro-NH_2 (4,5). TRH causes the rapid release of pituitary thyrotropin (TSH) both *in vivo* and *in vitro*. Consistent effects of TRH on the release of other pituitary hormones have not been described. In this report we present the results of experiments which show that TRH stimulates the production of prolactin and inhibits

growth hormone production in established strains of functional pituitary cells in culture.

MATERIALS AND METHODS

Synthetic TRH (Abbott, Lot No. 842-553) was dissolved in 0.15M NaCl, sterilized by filtration, and added to culture medium at concentrations described in each experiment. Three clonal strains of rat cells were used. They were the GH_3 and GH_1 strains of pituitary tumor cells (6), and the MH_1C_1 strain of hepatoma cells (7). Cells were grown at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, in 60 mm plastic tissue culture dishes. Medium was Ham's F 10 (8) supplemented with 15% horse serum and 2.5% fetal calf serum (3 ml/dish). Prolactin, growth hormone (GH), and serum albumin secreted into culture medium were measured by microcomplement-fixation immunoassay methods (6,7,9) which are specific for each antigen. The 95% confidence limits of a single determination are ± 20-25%; in most of the experiments reported at least duplicate culture dishes were used. Previous studies with these cell systems have shown that less than 5% of the specific proteins secreted by the cells are degraded in the medium per 24 hr (7,9-11). In addition, it has been shown that the pituitary cells in culture store very little intracellular prolactin (9) or GH (10)--amounts equal to those secreted into the medium in 1-2 hr or 15 min for prolactin and GH, respectively. Cell protein was determined by the method of Lowry et al. (12).

RESULTS

When GH_3 cells were grown in medium containing TRH, the rate of prolactin production* was greater than that in control cultures (Fig. 1). TRH-treated cells produced 2-5 times more prolactin than control cultures

*The quantity "rate of prolactin production" as used in this report is equal to the rate of appearance of prolactin in the medium (9-11). The amounts of prolactin (and GH) measured in the medium were divided by the total cell protein at the time of collection and then divided by the number of days the medium had been in contact with the cells. Thus, each reported value for hormone production represents the average specific rate of production per day throughout that collection period.

for a period of up to 20 days, the duration of this experiment. There was no effect of TRH on the rate of cell growth, and GH production was reduced about 30% by TRH (Fig. 1).

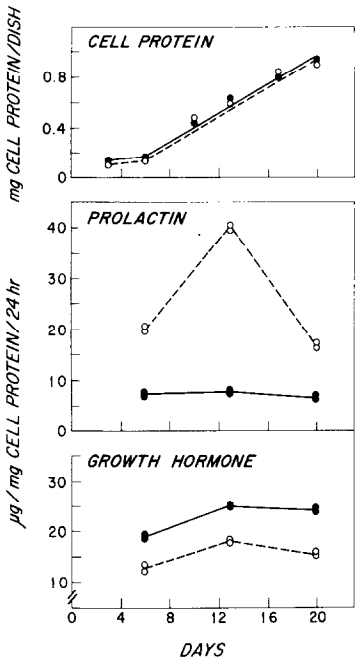

Fig. 1. Effects of TRH on cell protein, and on prolactin and GH production by cultures of GH_3 cells. At zero time, medium either containing TRH (10 ng/ml) or lacking TRH was added to each dish. Fresh medium either with or without TRH was added every 3 or 4 days. Medium was collected at intervals from duplicate experimental (0) and control (●) dishes and was frozen for hormone assays. At each medium change, a pair of experiment and control dishes was washed and frozen for determination of cell protein.

The time-course of the onset of the effect of TRH on prolactin and GH production is shown in Fig. 2. Within 4 hr a small but definite stimulation of prolactin production was observed; no effect on GH was detected at this early time interval. By 24 hr, the production of prolactin was markedly stimulated, and GH production was depressed.

Effects of various concentrations of TRH on prolactin production are shown in Fig. 3. At 24 hr, maximum stimulation was observed at a dose level of TRH of 10 ng/ml, and a significant (70%) stimulation was seen at 0.10 ng/ml. In view of the rapid inactivation of TRH by serum (2), the

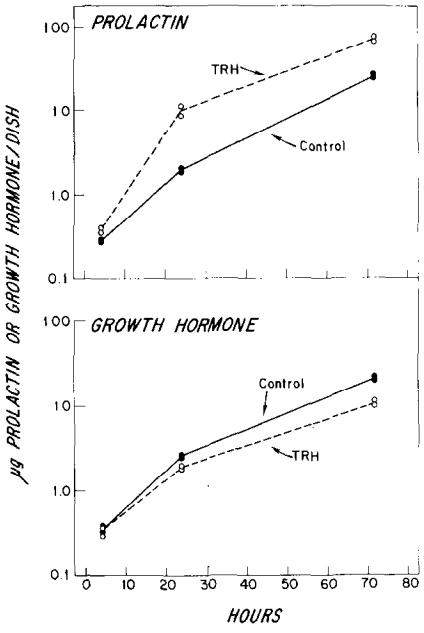

Fig. 2. Effects of treatment with TRH for 4, 24 and 72 hr on prolactin and GH production by GH_3 cells. At zero time, medium either lacking TRH or containing TRH (10 ng/ml) was added to control (●) or experimental (○) cultures. Medium was collected from duplicate dishes at 4, 24 and 72 hr and was frozen for hormone assays. There was no difference in cell protein between control (0.65 mg/dish) and experimental (0.65 mg/dish) cultures at 72 hr. Note log scale on the ordinate.

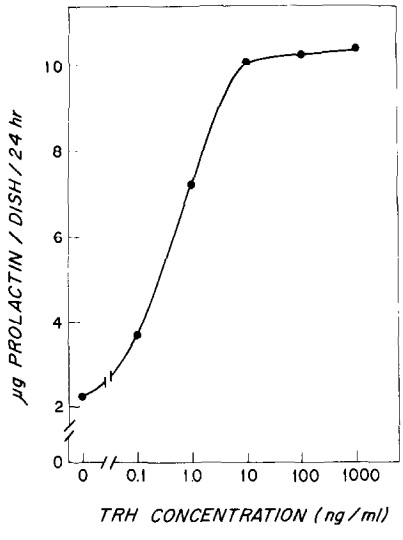

Fig. 3. Effects of various concentrations of TRH on prolactin production by GH_3 cells. Replicate dishes were incubated for 24 hr with TRH at the concentrations shown. Each point gives the mean of duplicate samples.

values given in Fig. 3 are probably minimum estimates of the sensitivity of GH_3 cells to TRH.

An experiment was performed to examine the effects of brief exposure to TRH on prolactin production. GH_3 cells were incubated for 1 hr in medium containing 10 or 100 ng TRH/ml or for 6 hr in medium containing 10 ng/ml. The medium was then removed, the cells were washed, and fresh medium lacking TRH was added and collected at 24 hr-intervals for 3 days. Exposure to 10 ng TRH/ml for 1 hr resulted in no stimulation of prolactin production; however, exposure to TRH for 1 hr at 100 ng/ml or 6 hr at 10 ng/ml stimulated prolactin production 2- to 3-fold within 24 hr, and the effect persisted for 3 days.

The GH_3 strain is not the only line of rat pituitary cells which responds to TRH. Table 1 shows that TRH also stimulates the production of prolactin and inhibits GH production in the GH_1 strain. On the other hand,

Table 1

EFFECTS OF TRH ON GH_1 PITUITARY CELLS AND MH_1C_1 HEPATOMA CELLS

Treatment	Dose	GH_1 cells		MH_1C_1 cells
		Prolactin	GH	Albumin
	(ng/ml)	(μg/mg cell protein/24 hr)		(μg/mg cell protein/24 hr)
Control	---	10	8.0	0.42
		11	7.7	0.39
TRH	10	20	5.9	0.45
TRH	100	15	4.2	0.38
TRH	1000	18	5.0	0.38

Conditions were similar to those described in Figs. 1 and 2. Rates of prolactin, GH and serum albumin production are given for 72-168 hr after beginning treatment with TRH. TRH did not affect cell protein in either GH_1 or MH_1C_1 cells.

TRH does not indiscriminately affect all differentiated functions in cells in culture as evidenced by the lack of effect on albumin production by the MH_1C_1 strain of rat hepatoma cells.

The integrity of the tripeptide structure of TRH is essential for its effect on GH_3 cells. Hydrolyzed TRH did not stimulate prolactin production (Table 2).

Table 2

EFFECT OF ACID HYDROLYSIS ON THE PROLACTIN-STIMULATING ACTIVITY OF TRH

Treatment	Dose (ng/ml)	Cell protein (mg/dish)	Prolactin production (µg/mg cell protein/24 hr)
Control	---	0.36	5.0 4.4
TRH	10	0.36	17
TRH	100	---	21
Hydrolyzed TRH	10	0.36	4.4
Hydrolyzed TRH	100	---	4.4
Hydrolyzed TRH	1000	0.38	4.7 5.0

TRH was hydrolyzed in 6N HCl at 110°C for 24 hr.

DISCUSSION

Results of the experiments described in this report show that TRH stimulates the production of prolactin by pituitary cells in culture. The effect on prolactin production was not merely the result of a nonspecific stimulation of protein synthesis because total cell protein was unaffected and GH production was reduced. Since the accumulation of prolactin in medium exceeded, by a factor of 10- to 50-fold, the amount of intracellular prolactin (9), TRH was not acting in these experiments only as a "releasing

factor". The observed findings could be the result of increased prolactin synthesis, decreased intracellular turnover, or a combination of these effects. However, since most of the prolactin and GH synthesized by GH_3 cells is secreted rapidly into the culture medium (9,10), thereby escaping those mechanisms which degrade intracellular enzymes and structural proteins, it is likely that the effects of TRH on prolactin and GH are due to increased and decreased synthesis, respectively. This hypothesis remains unproven, however, until further studies involving amino acid incorporation into each specific protein have been completed.

The primary if not the sole action of TRH has been considered to be stimulation of the release of TSH from the pituitary gland (1-3). Effects on pituitary biosynthetic pathways or on hormones other than TSH have not been observed regularly or measured in in vivo studies. Indeed, most previous evidence supports the view that each hypothalamic hypophysiotropic factor is specific for only one pituitary hormone (1-3). Results reported here suggest that a broader view of the actions of the hypophysiotropic hormones of the hypothalamus may be necessary. Our results show that TRH effects 2 hormones other than TSH. In addition, these effects, at least in the case of prolactin, may be mediated by alterations in the rate of hormone synthesis. However, interpretation of the physiological significance of these cell culture experiments must be cautious for several reasons: 1. the cells are neoplastic; 2. the concentrations of TRH, though low, may exceed those to which the pituitary gland is exposed in situ; and 3. the usual in vivo action of TRH on TSH is observed in minutes, whereas the effects described here occurred in hours to days. Nevertheless, we believe that functional pituitary cells in culture will play an important role in studies of the mechanisms and specificities of action of hypothalamic hormones.

ACKNOWLEDGMENTS

We thank Dr. M. S. Anderson of Abbott Laboratories, N. Chicago,

Illinois for the synthetic TRH. This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases (AM 11011).

REFERENCES

1. McCann, S. M. and Porter, J. C., Physiol. Rev. 49, 249 (1969).
2. Burgus, R. and Guillemin, R., Ann. Rev. Biochem. 39, 499 (1970).
3. Geschwind, I. I., In: Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry, J. Meites (ed.), Williams and Wilkins, Co., Baltimore, p. 298 (1970).
4. Burgus, R., Dunn, T. F., Desiderio, D. and Guillemin, R., C. R. Acad. Sci. (Paris) 269, 1870 (1969).
5. Folkers, K., Enzmann, F., Bøler, J., Bowers, C. Y. and Schally, A. V., Biochem. Biophys. Res. Commun. 37, 123 (1969).
6. Tashjian, A. H., Jr., Yasumura, Y., Levine, L., Sato, G. H. and Parker, M. L., Endocrinology 82, 342 (1968).
7. Richardson, U. I., Tashjian, A. H., Jr. and Levine, L., J. Cell Biol. 40, 236 (1969).
8. Ham, R. G., Expt. Cell Res. 29, 515 (1963).
9. Tashjian, A. H., Jr., Bancroft, F. C. and Levine, L., J. Cell Biol. 47, 61 (1970).
10. Bancroft, F. C., Levine, L. and Tashjian, A. H., Jr., J. Cell Biol. 43, 432 (1969).
11. Bancroft, F. C., Levine, L. and Tashjian, A. H., Jr., Biochem. Biophys. Res. Commun. 37, 1028 (1969).
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem. 193, 265 (1951).