PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 831–835 (1974)

Stimulation of Prolactin and Growth Hormone Release by TRH Infused into a Hypophysial Portal Vessel[1] (38200)

JIRO TAKAHARA, AKIRA ARIMURA, AND ANDREW V. SCHALLY

Department of Medicine, Tulane University School of Medicine, and Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Louisiana 70146

The administration of thyrotropin releasing hormone (TRH) to man, cattle, and sheep increases plasma prolactin levels as well as those of TRH (1–4). TRH also stimulates the secretion of GH in the rat (5, 6), cow (3), and in acromegalic and uremic human subjects (4, 7–10). Although the effect of TRH on the release of prolactin and GH in the rat was studied by several investigators (5, 6, 11–15), their results were inconsistent. During the purification of GH-releasing hormone (GH-RH) from porcine hypothamic extracts, we found that some purified fractions which contained TRH stimulated the release of both GH and prolactin in rats when they were infused into a hypophysial portal vessel (16). It was necessary to clarify whether or not GH and prolactin releasing activity in these fractions is due to TRH, to distinct GH-releasing and prolactin releasing hormones, or both. Consequently, the present study was undertaken to investigate the effect of infusion of synthetic pure TRH into a hypophysial portal vessel on the release of GH and prolactin in the rat.

Materials and Methods. Male rats of the Sprague-Dawley strain weighing 250–350 g were used in this study. Some rats were injected with 50 μg estradiol in oil 18–24 hr before the experiments. The anterior pituitary and the pituitary stalk were exposed by a transpharyngial approach (17) under anesthesia with urethane (175 mg/100 g body weight ip). The test sample in 60 μl of 0.9% saline was infused into a hypophysial portal vessel for a 30 min period as described by Porter *et al.* (17) and Sandow *et al.* (18). Blood was collected from the jugular vein before and 30 and 60 min after the initiation of the infusion. Serum was separated by centrifugation and stored at −20° until assayed for GH and prolactin. The GH and prolactin releasing activity of samples was expressed as the net increase or the percentage increase of respective hormone in the serum at the time of termination of infusion as compared with the preinfusion levels.

Synthetic TRH was supplied by Abbott Laboratories. Serum prolactin was determined by radioimmunoassay (RIA) as described by Niswender *et al.* (19) using NIAMD-Rat-Prolactin Kit. Rat GH in serum was also measured by RIA (20), using NIAMD-Rat-GH-RIA Kit. Duncan's new multiple range test (21) was used to determine the significance of differences in prolactin releasing and GH releasing activities between control and experimental samples.

Results. As shown in Table I, the administration of 20 or 40 ng TRH did not raise serum prolactin. However, 100 ng and 1000 ng TRH significantly increased prolactin levels, but no dose-response relationship was observed. Pretreatment with estradiol increased the basal serum prolactin level, and had a tendency to augment prolactin release induced by TRH, but the difference was not statistically significant because of the large variation. On the other

[1] This study was supported by Veterans Administration, USPHS Research Grants AM-07467 and AM-09094.

TABLE I. Effect of Infusion of Synthetic TRH into a Hypophysial Portal Vessel on Prolactin Release in Male Rats.

Treatment	Sample	Dose (ng/60 μl)	No. of rats	Serum prolactin (ng/ml) [a]			30 min × 100% / Preinfusion	P Value vs control
				Preinfusion	30 min	Net increase		
	Control (0.9% Saline)	—	5	57.8 ± 3.7[b]	56.1 ± 2.9	−1.7 ± 3.9	98.9 ± 7.3	—
—	Synthetic	4	5	64.0 ± 12.5	63.1 ± 18.6	−0.9 ± 8.8	95.2 ± 15.3	NS[c]
		20	7	66.5 ± 13.0	68.6 ± 14.5	2.1 ± 13.9	114.4 ± 22.2	NS
	TRH	100	8	50.7 ± 9.6	88.6 ± 10.2	38.0 ± 8.9	200.3 ± 32.0	$P < 0.01$
		1000	8	55.0 ± 14.2	91.4 ± 24.7	36.5 ± 11.0	176.9 ± 21.0	$P < 0.01$
50 μg Estradiol in oil	Control	—	5	155.6 ± 53.6	120.8 ± 40.4	−22.4 ± 13.2	77.6 ± 13.2	—
	Synthetic TRH	100	7	193.7 ± 28.3	251.3 ± 55.6	57.7 ± 43.9	128.0 ± 19.1	NS

[a] Expressed as NIAMD-Rat-Prolactin-RP-1.
[b] Mean ± S.E.
[c] Not significant.

TABLE II. Effect of Infusion of Synthetic TRH into a Hypophysial Portal Vessel on GH Release in Male Rats.

Treatment	Sample	Dose (ng/60 μl)	No. of Rats	Serum GH (ng/ml) [a]			P Value vs control
				Preinfusion	30 min.	Net increase	
	Control (0.9% Saline)	—	5	11.4 ± 4.5[b]	13.6 ± 6.6	2.3 ± 6.9	—
	Synthetic	4	5	16.0 ± 9.3	30.8 ± 10.3	14.8 ± 14.2	NS[c]
		20	7	6.6 ± 2.8	37.0 ± 10.9	30.3 ± 9.8	NS
	TRH	100	8	14.6 ± 5.5	63.5 ± 18.3	45.3 ± 18.1	$P < 0.05$
		1000	8	18.6 ± 5.8	170.3 ± 54.6	161.7 ± 53.5	$P < 0.01$
50 μg Estradiol in oil	Control	—	5	31.2 ± 5.5	24.5 ± 4.4	−6.7 ± 4.3	—
	Synthetic TRH	100	7	49.3 ± 11.9	63.6 ± 7.5	12.8 ± 13.6	NS

[a] Expressed as NIAMD-Rat-GH-RP-1.
[b] Mean ± S.E.
[c] Not significant.

hand, 4 and 20 ng of TRH appeared to increase serum GH, but the increment was not significant. A significant rise of serum GH was induced by 100 ng and 1000 ng TRH, and the response was dose-related. As in the case of prolactin, estradiol pretreatment also raised the basal level of serum GH, but the net GH increase in response to TRH was diminished.

Discussion. The results show that fairly large doses of synthetic TRH infused into a rat hypophysial portal vessel raised the serum levels of both GH and prolactin, suggesting a direct stimulation of the release of these hormones by TRH. Previously, it was reported that intravenous injection of TRH caused a significant increase in plasma prolactin level in proestrous rats (5, 6) and estrogen primed male rats (6). On the other hand, lack of response to TRH was also reported by other investigators (12, 21). No report on the effect of TRH on GH release *in vivo* in rats has appeared yet.

Convey *et al.* (3) reported that the iv administration of TRH increased serum prolactin and GH levels in cattle. However, they failed to observe stimulation of prolactin release by TRH from bovine pituitary tissue *in vitro*. They proposed that TRH may act on the hypothalamus and release GH-RH and PRH which in turn influences the pituitary. However, recently, we observed that addition of TRH to the incubation medium stimulated the release of prolactin and GH from sheep pituitary fragments *in vitro* (Takahara *et al.*, in preparation). Tashjian *et al.* (11) found that TRH inhibited GH secretion by cloned cell lines of a rat pituitary tumor in tissue culture. Wilber *et al.* (14) observed no effect of TRH on GH secretion by rat hemipituitaries incubated *in vitro*. In contrast, Carlson *et al.* (10) and Machlin *et al.* (15) reported that TRH increased GH release from rat pituitary explants *in vitro* and from cultured bovine pituitary cells. In our experiment, the infusion of 100 ng and 1000 ng of TRH into a hypophysial portal vessel caused a significant increase in serum GH levels, and the response appeared to be dose-related. Previous reports as well as the results in the present study suggest that under certain conditions TRH stimulates prolactin and GH release by direct action on the anterior pituitary, although there are species differences in the sensitivity of GH and prolactin cells to TRH.

It has been reported that the secretory response of the anterior pituitary to some stimuli and to hypothalamic hormones is augmented after pretreatment of estrogen (22, 23, 26, 27). Bowers *et al.* (1) and Jacobs *et al.* (24) observed a greater increase in serum prolactin concentration in response to TRH in women than in men. This phenomenon could be, at least in part, attributed to the influence of estrogens. In the present study, pretreatment with estrogen elevated the basal serum prolactin levels, but the net increase of GH in prolactin after TRH remained unchanged or rather decreased. Since the absolute values of serum GH and prolactin after TRH in estrogen-primed rats were the same or larger than those in the control rats, the lack of augmentation of the pituitary response after estrogen pretreatment may be simply due to the elevated preinfusion values. When the hypophysial portal vessel infusion technique is used, a given dose of TRH may induce the maximum GH response and elevation of the basal secretion by estrogen pretreatment results in a decrease in the net release of GH.

Summary. The effects of infusion of synthetic thytropin releasing hormone (TRH) into a hypophysial portal vessel on the release of prolactin and growth hormone (GH) were investigated in normal male rats under urethane anesthesia. Significant increases in serum prolactin and GH levels were consistently observed after the infusion of 100 ng or larger doses of synthetic TRH, but smaller doses had no effect. Pretreatment of rats with 50 μg estradiol in oil raised the basal levels of prolactin and GH, but the net increase after TRH remained the same for prolactin and was somewhat reduced for GH.

The authors are indebted to Mrs. J. Gauthier and Mrs. M. Esslinger for their excellent technical assistance, Dr. Abba J. Kastin for his help in

preparing the manuscript, and NIAMD Rat Pituitary Hormone Program for the supply of rat GH and Prolactin RIA Kits.

1. Bowers, C. Y., Friesen, H. G., Hwang, P., Guyda, H. J., and Folkers, K., Biochem. Biophys. Res. Commun. **45**, 1033 (1971).

2. Jacobs, L. S., Synder, P. J., Utiger, R. D., Wilber, J. F., and Daughaday, W. H., J. Clin. Endocrinol. Metab. **33**, 996 (1971).

3. Convey E. M., Tucker, H. A., Smith, V. G., and Zolman, J., Endocrinology **92**, 471 (1973).

4. Rakoff, J. S., Siler, T. M., Sinha, Y. N., and Yen, S. S. C., J. Clin. Endocrinol. Metab. **37**, 641 (1973).

5. Deis, R. P., and Alonso, N., J. Endocrinol. **58**, 673 (1973).

6. Mueller, G. P., Chen, H. J., and Meites, J., Proc. Soc. Exp. Biol. Med. **144**, 613 (1973).

7. Irie, M., and Tsushima, T., J. Clin. Endocrinol. Metab. **35**, 97 (1972).

8. Schalch, D. S., Gonzalez-Barcena, D., Kastin, A. J., Schally, A. V., and Lee, L., J. Clin. Endocrinol. Metab. **35**, 609 (1972).

9. Gonzalez-Barcena, D., Kastin, A. J., Schlach, D. S., Torres-Zamora, M., Perez-Pasten, E., Kato, A., and Schally, A. V., J. Clin. Endocrinol. Metab. **36**, 117 (1973).

10. Carlson, H. E., Mariz, I. K., and Daughaday, W. H., Abstract of the 55th Annual Meeting of the American Endocrine Society, p. 141 (1973).

11. Tashjian, A. H., Jr., Barawsky, N. J., and Jensen, D. K., Biochem. Biophys. Res. Commun. **43**, 516 (1971).

12. Lu, K. H., Shaar, C. J., Kortight, K. H., and Meites, J., Endocrinology **91**, 1540 (1972).

13. Vale, W., Blackwell, R., Grant, G., and Guillemin, R., Endocrinology 93, 26 (1973).

14. Wilber, J., Peake, G., Mariz, I., Utiger, R., and Daughaday, W. H., Clin. Res. **16**, 277 (1968).

15. Machlin L. J., and Jacobs, L. S., Abstract of the 55th Annual Meeting of the American Endocrine Society, p. 243 (1973).

16. Takahara, J., Arimura, A., and Schally, A. V., Neuroendocrinology, submitted, (1973).

17. Sandow, J., Arimura, A., and Schally, A. V., Endocrinology **90**, 1315 (1972).

18. Porter, J. C., Mical, R. S., Kamberi, I. A., and Grazia, Y. R., Endocrinology **87**, 197 (1970).

19. Niswender, G. D., Chen, C. L., Midgley, A. R., Jr., Meites, J., and Ellis, S., Proc. Soc. Exp. Biol. Med. **130**, 793 (1969).

20. Schalch, D. S., and Reichlin, S., Endocrinology **79**, 275 (1966).

21. Steel, R. D. G., and Torrie, J. H., *in* "Principles and Procedures of Statistics," p. 107. McGraw-Hill Book Co. (1966).

22. Valverde, R. C., and Chieffo, V., Prog. 53rd Meeting Endocrine Society, June 24–26 (1971) San Francisco.

23. LaBella, F. S., and Vivian, V., Endocrinology **88**, 787 (1971).

24. Jacobs, L. S., Synder, P. J., Utiger, R. D., and Daughaday, W. H., J. Clin. Endocrinol. Metab. **36**, 1069 (1973).

25. Carlson, H. E., Jacobb, L. S., and Daughaday, W. H., J. Clin. Endocrinol. Metab. **37**, 488 (1973).

26. Frantz, A. G., and Rablin, M. T., J. Clin. Endocrinol. Metab. **25**, 1470 (1965).

27. Merimee, T. J., Burgess, J. A., and Rabinowitz, D., J. Clin. Endocrinol. Metab. **26**, 791 (1966).

Received Mar. 13, 1974. P.S.E.B.M., 1974, Vol. 146.