Effect of TRH and Triiodothyronine on Prolactin Release in Sheep[1] (37037)

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The regulation of pituitary TSH secretion by the hypothalamus is mediated by TRH: (pyro)Glu-His-Pro-NH_2 (1, 2) and administration of synthetic TRH elicits TSH release in animals and man (3). It was recently reported that TRH released prolactin from rat pituitary *in vitro* (4). The present paper describes our recent observations on the release of prolactin by TRH and the blockade of this effect by pretreatment with triiodothyronine (T_3) in sheep.

Materials and Methods. Three whitefaced anestrous ewes and one vasectomized ram maintained under cycles of 16 hr light and 8 hr darkness were used throughout the experiment. Each sheep had been previously prepared with a carotid loop to facilitate intracarotid injections (5). In order to bleed the animals without discomfort, cannulae were kept in the jugular veins. Two control blood samples were taken 15 min apart from each animal. Two milliliters of normal saline were injected over a period of 2 min into the carotid artery and blood was taken from the jugular vein 5, 20, 40, and 60 min after the injection. Synthetic TRH (0.5 mg/sheep) in 2 ml saline solution was then injected into the carotid artery over a period of 2 min and blood was collected from the jugular vein 5, 10, 25, 45, and 60 min afterwards. Blood samples were refrigerated overnight, centrifuged, and the serum kept frozen until assayed.

In another study one anestrous ewe and one vasectomized ram were used. After a control bleeding, the animals were injected sc with 3 mg of T_3 in 0.5 ml of 0.1 N sodium hydroxide solution. One and 2 hr later the animals were bled again. TRH in 2 ml saline solution was then administered by intracarotid injection (0.5 mg/sheep) and blood was taken from the jugular cannula 5, 10, 25, 45 and 60 min later. Prolactin, GH, TSH and LH in each sample were determined.

Prolactin was assayed in duplicate by the double antibody radioimmunoassay described by Arai and Lee (6) and modified by Reeves *et al.* (7). NIH prolactin-S_8 was used as the standard preparation. Purified ovine prolactin was used for labelling with ^{125}I and guinea pig anti-ovine prolactin serum supplied from Dr. Lee was used as the first antibody. ^{125}I-prolactin was diluted with 1% bovine serum albumin so that 100 μg gave 6000 to 7000 cpm. An excellent standard curve was obtained within a range of 2 to 64 ng/ml of prolactin. No cross-reactions with ovine LH, FSH, GH, TSH, or TRH were observed. Serum LH was measured in duplicate by means of a double antibody radioimmunoassay method (7), using NIH-LH-S_{17} as standard, purified ovine LH (supplied by Dr. Papkoff) for iodination with ^{125}I, and guinea pig anti-ovine LH serum (supplied by Dr. White). Serum GH was measured by a double antibody radioimmunoassay (8). Serum TSH was determined by the bioassay described by McKenzie (9).

Results. Injection of saline did not significantly raise serum prolactin or LH levels (Table I). However, the injection of TRH induced a prompt and significant rise in serum prolactin. The maximal increase was observed 5 and 10 min after the injection of TRH and then the level fell slowly, still being higher than the control values 60 min

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TABLE I. The Effect of TRH on Serum TSH, Prolactin, GH and LH Levels in 3 Anestrous Ewes and 1 Vasectomized Ram.

	TSH (a)		Prolactin (b)		GH (c)		LH (d)	
	M	F	M	F	M	F	M	F
Control 1	ND	ND[a]	275	83.8 ± 16.7	30.4	4.5 ± 1.1	8.4	0.56 ± 0.01
Control 2	ND		287.5	75.0 ± 19.8	25.6	6.8 ± 2.4	7.1	0.37 ± 0.1
Injection of Saline								
5 minutes	ND		270	74.8 ± 24.9	28.8	3.2 ± 1.2	7.1	0.75 ± 0.1
20 minutes	ND	ND	255	61 ± 16.5	26	5.0 ± 0.2	7.3	ND
40 minutes	ND	ND	262.5	66.6 ± 23.3	23.6	1.8 ± 1.4	7.0	0.35 ± 0.1
60 minutes	ND	ND	280	77.5 ± 24.6	24.8	3.3 ± 1.5	6.6	2.3 ± 2
Injection of TRH								
5 minutes			1600	1474.1 ± 76.7	41.2	5.2 ± 2.2	9.1	1.96 ± 0.6
10 minutes	ND	1.5(e)	1600	1086.6 ± 120.6	31.6	2.6 ± 1.1	8.1	1.28 ± 0.3
25 minutes			1600	629.1 ± 89.9	28.4	2.5 ± 0.1	9	0.76 ± 0.4
45 minutes	0.215	0.7(e)	1175	368.3 ± 17.4	26.4	2.8 ± 0.8	8.05	1.8 ± 0.5
60 minutes			1050	389.1 ± 64.3	26.8	3.2 ± 0.1	7.2	1.5 ± 0.7

M Male
F Female
(a) Expressed as mU NIH-TSH-B_5/ml
(b) Expressed as ng NIH-Prolactin-S_8/ml
(c) Expressed as ng NIH-GH-S_8/ml
(d) Expressed as ng NIH-LH-S_{17}/ml
(e) TSH assay could be performed only in one sheep
[a] Not detectable.

after injection. Serum TSH rose from undetectable to measurable levels after the injection of TRH, but the serum LH and GH levels did not change.

Administration of T_3 lowered resting serum prolactin levels in both animals studied (Table II) but after injection of TRH, serum prolactin rose to levels similar to those prior to T_3 treatment. These levels remained practically unchanged during the following 1 hr period. TSH was not detectable in any of the samples. Serum GH levels did not change throughout this experiment.

Discussion. The results of these experiments are in agreement with the reports by other investigators that TRH releases prolactin as well as TSH in rats and man (4, 10). The mechanism by which TRH releases prolactin is unknown. Treatment with T_3 before the injection of TRH prevented the rise in both serum TSH and prolactin. This indicates that the effect of T_3 is intimately related to the prolactin-releasing activity of TRH as well as to the TSH-releasing activity. Furthermore, it has been demonstrated recently that thyroidectomy stimulates, and treatment with T_3 inhibits, prolactin synthesis in the rat (11). TRH may influence the mechanisms which control prolactin secretion by acting directly on the pituitary or, alternatively, on the hypothalamus. The first possibility, however, is more likely, since Tashjian *et al.* (4) demonstrated that TRH releases prolactin when incubated directly in contact with pituitary cells. Moreover, it is well known that T_3 inhibits TRH-induced release of TSH by acting directly on the pituitary gland. In these experiments the suppression by T_3 of a TRH effect on prolactin and TSH release also favors the possibility of a direct action on the pituitary gland.

At present it is not known whether the prolactin-releasing effect of TRH is a physiological mechanism. In the present investigation the dose of TRH used was probably in the pharmacological range.

Summary. The intracarotid injection of TRH, sufficient to increase serum TSH, brought about a considerable rise in serum prolactin levels in three female anestrous ewes and one vasectomized male ram. The peak in serum prolactin was seen 5 and 10

TABLE II. The Effect of T_3 On the Response to TRH in an Anestrous Ewe and a Vasectomized Ram.

	Time in minutes							
	Inj. of T_3			Inj. of TRH				
	−120	−60	0	5	10	25	45	60
TSH (a) Female	ND[a]		0.62	ND	ND	ND	ND	ND
Male	ND		ND	ND	ND	ND	ND	ND
Prolactin (b) Female	140	12.5	10	140	165	100	72.5	92.5
Male	350	185	200	355	332.5	305	290	328
GH (c) Female	2.0	0.4	0.4	2.4	9.2	ND[a]	2.0	0.8
Male	17.2	18.4	20.8	21.2	20	18.8	21.2	21.2
LH (d) Female	0.95	2.6	6.6	1.2	1.5	0.9	0.4	0.5
Male	10.0	11.25	13.0	11.4	10.2	10.2	10.1	10.8

(a) Expressed as mU NIH-TSH-B_6/ml
(b) Expressed as ng NIH-Prolactin-S_8/ml
(c) Expressed as ng NIH-GH-S_8/ml
(d) Expressed as ng NIH-LH-S_{17}/ml
[a] Not detectable.

min after injection of TRH; then the levels declined, but they were still elevated 60 min later. Pretreatment of the sheep with T_3 inhibited the rise in serum prolactin as well as serum TSH after injection of TRH.

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1. Schally, A. V., Redding, T. W., Bowers, C. Y., and Barrett, J., J. Biol. Chem. **244**, 4077 (1969).

2. Burgus, R., Dunn, T. F., Desiderio, D., and Guillemin, R., Cr. Seanc. Acad. Sci. Paris **269**, 1870 (1969).

3. Schally, A. V., and Bowers, C. Y., Proceedings 6th Midwest Conference on the Thyroid and Endocrinology, p. 25, October 2–3, 1970, University of Missouri, Columbia, Missouri.

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

5. Bone, J. F., Metcalfe, J., and Pares, J. T., Amer. J. Vet. Res. **23**, 113 (1962).

6. Arai, Y., and Lee, T. H., Endocrinology **81**, 1941 (1967).

7. Reeves, J. J., Arimura, A., and Schally, A. V., Proc. Soc. Exp. Biol. Med. **134**, 938 (1970).

8. Morgan, A. C., and Lazarow, P., Diabetes **12**, 115 (1963).

9. McKenzie, J. M., Endocrinology **63**, 372 (1958).

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

11. Ieiri, T., Jap. J. Physiol. **21**, 551 (1971).

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