In Vivo Stimulation of Prolactin Release in the Rat by Synthetic TRH[1,2] (37645)

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Synthetic thyrotropin-releasing hormone (TRH), pyroglutamylhistidylproline amide, stimulates release of thyroid-stimulating hormone (TSH) in several species *in vivo* and *in vitro* (1, 2). Tashjian *et al.* (3) observed that TRH increased prolactin release when added to cultures or short-term incubations of clonal cells from rat pituitary tumors, but TRH was not clearly shown to stimulate *in vitro* release of prolactin from normal rat hemi-pituitaries (4, 5) or from incubated bovine pituitary tissue (6, 7). TRH stimulated *in vivo* release of prolactin in the human (8, 9) and in the bovine (7, 10) but apparently not in the rat (4). This study was undertaken to further explore the possibility that TRH may induce *in vivo* release of prolactin in the rat, and to examine the relation of estrogen to TRH induced prolactin release.

Materials and Methods. Mature Sprague–Dawley virgin female and male rats (Spartan Research Animals, Haslett, Mich.), 3–4 months old, were housed in an air-conditioned (74 ± 2°F) and light controlled (lights on from 5 AM–7 PM) room. They were fed a diet of Wayne Lab Blox pellets (Allied Mills, Chicago, Ill.) and provided with water *ad libitum*. Daily vaginal smears were taken from female rats for at least two recurrent 4- or 5-day estrous cycles to insure that they were undergoing regular cycles. On the morning of proestrous, a pretreatment blood sample was collected from the orbital sinus under light ether anesthesia at about 11:00 AM, and immediately thereafter the animals were injected iv with 200 ng, 500 ng, 1 μg, 5 μg, or 25 μg of synthetic TRH.[3] In all cases the injection volume was 0.2 ml of 0.87% NaCl and the controls were given NaCl alone. Posttreatment samples were collected 10 and 60 min after injection. The male rats were given sc injections of either 10 μg estradiol benzoate (Nutritional Biochemicals Corp., Cleveland, Ohio) dissolved in 0.2 ml corn oil or corn oil alone for 5 days. On the sixth day a pretreatment blood sample was collected at about 11:00 AM and the animals were immediately injected iv with either 0.2 ml NaCl or 1 μg TRH dissolved in 0.2 ml NaCl. Posttreatment samples were collected at the end of 10 and 60 min. Serum was separated and stored at −20° until assayed for prolactin by radioimmunoassay (11). Prolactin values are expressed in terms of NIAMD-Rat Prolactin-RP-1. Student's *t* test was used to determine significance of differences between any 2 groups.

Results. All doses of TRH used evoked a 2–3.5-fold increase in serum prolactin by 10 min after injection into proestrous rats as compared with control rats (Table I). The 1-μg dose produced the greatest increase in serum prolactin 10 min after injection when compared with control values, and was slightly more effective than the other doses of TRH when compared with pretreatment values. By 60 min after TRH injection serum prolactin levels fell but were still higher than pretreatment values. There appeared to be no definite dose-response relationship observed between the doses of TRH

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TABLE I. Effects of TRH on Prolactin Release in Proestrous Female and in Untreated and Estrogen-Primed Male Rats.

Treatment (no. rats)	Serum prolactin* (ng/ml ± SE of mean)		
	Pretreatment	10 min	60 min
Proestrous female rats			
Controls (14)	44 ± 9	68 ± 12	72 ± 12
200 ng TRH (11)	42 ± 11	127 ± 24[a]	73 ± 16
500 ng TRH (10)	63 ± 15	187 ± 25[c]	107 ± 22
1 μg TRH (9)	61 ± 17	222 ± 26[c]	73 ± 12
5 μg TRH (9)	42 ± 13	132 ± 18[b]	68 ± 16
25 μg TRH (10)	51 ± 12	142 ± 23[b]	127 ± 24[a]
Male rats			
Controls, no estrogen (9)	15 ± 2	45 ± 8	32 ± 4
1 μg TRH, no estrogen (9)	20 ± 4	73 ± 8[a]	46 ± 7
Controls, estrogen (9)	106 ± 9	113 ± 5	146 ± 11
1 μg TRH, estrogen (10)	120 ± 9	262 ± 11[c]	216 ± 20[a]

* Controls vs experimental group at same time period; [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

given and the increases in serum prolactin.

In both untreated and estrogen-primed male rats, significant increases in serum prolactin were observed after injection of 1 μg TRH. The pretreatment control, nonestrogen-primed male rats had less serum prolactin than the control female rats, and estrogen markedly increased serum prolactin levels.

Discussion. This study shows that a single iv injection of synthetic TRH can stimulate a rapid 2.0–3.5-fold rise in serum prolactin in proestrous female rats and in normal and estrogen-primed male rats. Previously our laboratory reported that a single iv injection of 7.5 μg TRH or intracarotid infusion of 5 μg TRH failed to increase serum prolactin by 15 or 30 min after administration (4). This may be due to the different preparation of TRH used and the longer interval that elapsed after injection, before blood samples were collected. Unpublished observations from our laboratory suggest that the peak rise in serum prolactin in female rats occurs in less than 10 min after TRH injection. In the bovine, maximum increases in blood prolactin were observed 6–10 min after TRH injection (7, 10). The increase in serum prolactin in each of the control groups with time is believed to reflect the stress associated with multiple blood collections and anesthesia (12).

Valverde *et al.* (13) reported no rise in serum prolactin in estrogen-primed male rats after injection of 100 ng TRH, but this dose may be insufficient to produce an increase in prolactin release. The TRH dose employed in the present study in male rats was 10 times as great, and was effective in both estrogen-primed and nonestrogen-primed male rats. However, proestrous female rats showed a greater prolactin increase in response to TRH injection than the estrogen-primed male rats, suggesting that factors other than estrogen may influence this response. The present observations indicate that rats react to *in vivo* administration of synthetic TRH similarly to the human (8, 9) and cow (7, 10).

Summary. Synthetic TRH (iv) can significantly increase serum prolactin in proestrous female and in normal and estrogen-primed male rats by 10 min after injection.

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