FURTHER EVIDENCE THAT TRH IS ALSO A PHYSIOLOGICAL REGULATOR OF PRL SECRETION IN MAN

by

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SUMMARY From results of the effect of synthetic pyroglutamyl-histidyl-prolineamide, pGlu-His-ProNH2 or TRH, in normal women and men the most compelling indirect evidence has been obtained which supports the hypothesis that TRH may be a physiological regulator of both TSH and PRL. The minimum effective dose of TRH which stimulates TSH and PRL release in normal men and women is essentially the same. After the administration of TRH to normal subjects, there was always an increase of PRL as well as TSH. The proposed term prolactin-thyrotropin releasing hormone or PTRH rather than TRH may more precisely indicate the biological activities of pGlu-His-ProNH$_2$ in man.

The recent isolation of prolactin (PRL) from the human pituitary along with the development of a very sensitive and specific radioimmunoassay (RIA) for measuring PRL in human serum has made it possible to study the mechanisms involved in the production and secretion of this hormone (1-3). Animal studies as well as certain clinical observations in man have clearly established that the hypothalamus regulates the secretion of PRL (4-7). The control of PRL secretion in man appears to have many similarities to that found in animals. For example, in both animals and man after section of the pituitary stalk and administration of chlorpromazine, serum PRL levels rise (8). Presumably this increase results from decreased secretion of prolactin inhibiting factor or hormone (PIF or PIH) from the hypothalamus. Data reported by Nicoll and Meites (9), Krulich et al (10), Valverde and Chieffo (11), and Valverde et al (12), suggests that the hypothalamus also contains a substance designated prolactin releasing factor or hormone (PRF or PRH) which stimulates prolactin release. The chemical nature of

this factor is unknown; however, Tashjian's (13) observation that TRH stimulates PRL production in vitro in clonal strains of rat pituitary cells raised the possibility that TRH might have a dual role regulating the secretion of both TSH and PRL. Indeed, pGlu-His-ProNH$_2$ stimulates the release of PRL and TSH in man (14,15,16) and in monkeys (17). Furthermore, if serum was added to the incubation medium, TRH was found to stimulate release of both TSH and PRL from the pituitary of the monkey (unpublished).

Because the minimal effective dose of TRH which was required to release TSH or PRL was identical, we suggested that this hypothalamic hormone may stimulate the release of both TSH and PRL under certain physiological circumstances (14). The present studies have been performed to more critically evaluate the possibility of whether pGlu-His-ProNH$_2$ is a physiological regulator of both PRL and TSH in women and men.

METHODS: TRH was synthesized as previously described (18) and all hormones were measured in duplicate by radioimmunoassay methods (1, 19). TRH dissolved in 1 ml normal saline was injected intravenously within 15 to 20 seconds into ambulatory subjects after collection of the control blood sample. Each subject received saline, 10, 25, and 800 μg TRH at various times within a 8 month period. The ages of ten normal women ranged from 23-48 years (mean ±SE 32±2.6), and their serum T4 levels (normal 4-10) ranged from 5.9-7.9μg% (mean ±SE 6.9±.20). The ages for the ten normal men ranged between 21-47 years (mean ±SE 30±2.7), serum T_4 5.5-7.6μg% (mean ±SE 6.7±.24) and for the ten women with thyroid disorders 26-53 years (mean ±SE 45±3.8) and serum T_4 5.0-7.5μg% (mean ±SE 6.2±.31).

RESULTS: Tables 1-A and 1-B show the results of the TSH and PRL responses after the rapid intravenous injection of TRH. The responses obtained in ten normal women and men and in ten women with thyroid disorders are shown 15 and 30 minutes after the administration of TRH or saline. In all instances, 10, 25 and 800μg of TRH stimulated more TSH and PRL release than saline. The maximum PRL and TSH increase generally occurred at 15 minutes although in several instances the maximal TSH concentration was found at 30 minutes.

The results recorded in Tables 1-A and 1-B demonstrate that the TSH and PRL responses were significantly greater in the normal women than the normal men at 2 of the 3 doses of TRH administered. Furthermore, the PRL rise (Δ mμg/ml) was greater than the TSH rise (Δ μU/ml) in both normal women and men if we assume that 1μU TSH is equivalent to a weight of 25 picograms. Although the TSH and PRL responses were essentially the same after administration of 25 and 800μg of TRH to men, in women 800μg in comparison with 25μg, released twice as much of both TSH and PRL. Thus, the TSH and PRL dose-response range of TRH is greater in women than men but importantly, the TRH-TSH and PRL dose-response ranges

Table 1-A pGLU-HIS-PRO-NH_2(TRH) EFFECT ON TSH AND PRL RELEASE

Dose TRH μg	TSH μU/ml ±SE minutes			PRL mμg/ml ±SE minutes		
	0	15	30	0	15	30
Normal Women						
0	2.9±.38	3.0±.46	2.9±.37	9.8±1.9	10.8±2.8	9.4±2.3
10	3.5±.40	14.7±1.4	11.8±2.2	14.2±2.7	35.7±6.0	26.4±3.9
25	3.9±.52	22.4±4.1	19.3±3.2	12.4±3.9	48.5±8.5	35.3±8.1
800	4.0±.56	34.7±8.3	37.7±8.9	12.2±2.5	74.7±13.1	62.7±12.8
Normal Men						
0	2.6±.11	2.6±.11	2.5±.04	9.9±1.7	12.9±2.1	10.9±1.8
10	3.6±.73	9.1±1.4	9.4±1.6	10.2±1.6	21.0±2.2	19.6±2.2
25	3.6±.65	14.4±3.6	13.9±3.5	10.7±1.6	28.7±3.2	21.0±2.1
800	2.8±.27	12.7±2.8	15.5±3.5	10.2±1.7	33.9±4.6	27.4±3.5
Women with Thyroid Disorders						
0	36.0±8.6	34.7±7.9	35.0±8.3	9.0±1.0	9.7±.62	8.6±.83
10	28.9±5.7	65.3±11.5	58.2±9.9	8.9±1.3	40.3±5.3	32.1±5.9
25	40.6±9.9	122.0±24.5	118.0±24.2	6.5±1.1	52.0±6.0	42.3±7.0
800	37.6±7.8	129.0±19.9	152.0±22.3	9.2±2.5	77.1±14.1	71.4±18.1

At various times over a 6 month period saline and 10, 25 and 800μg TRH was administered as a quick iv injection at 0 time to each subject. Ten normal women and men and ten women with thyroid disorders were in each group. Each result is the mean of ten values ±SE.

Table 1-B pGLU-HIS-PRO-NH_2(TRH) EFFECT ON TSH AND PRL RELEASE

Dose TRH μg	TSH Δ mean* value	±SE	p value vs 0	p value vs 10	PRL Δ mean* value	±SE	p value vs 0	p value vs 10
Normal Women								
0	0.14	0.1	-	-	1.5	1.7	-	-
10	11.90	2.6	<.001	-	17.4	4.3	.010	-
25	18.90	3.7	<.001	ns	31.5	6.1	<.001	ns
800	31.40	9.1	<.001	.05	66.0	13.4	<.001	<.01
Normal Men								
0	0.10	.07	-	-	3.9	1.1	-	-
10	5.70	1.1	<.001	-	12.4	2.5	<.010	-
25	11.70	3.1	<.001	<.10	18.5	2.5	<.001	ns
800	12.60	3.1	<.001	<.05	22.5	4.0	<.001	<.001
Women with Thyroid Disorders								
0	-1.30	1.5	-	-	0.5	.97	-	-
10	36.00	7.4	<.001	-	31.4	4.8	<.001	-
25	83.00	17.7	<.001	<.020	54.0	6.2	<.001	<.01
800	116.00	17.8	<.001	<.001	63.0	15.5	<.001	<.05

Δ mean values are the maximal differences calculated from the serum hormonal levels of ten subjects before and 15 or 30 minutes after iv TRH.

parallel one another. The minimum effective dose (about 10μg) found to stimulate TSH and PRL release in normal men and women is essentially the same.

All of the ten women with thyroid disorders had previously had thyrotoxicosis, except one with a non-toxic goiter, and had been treated with radioiodine or surgery (Table 1-A and 1-B). These women were clinically euthyroid and were receiving no thyroid therapy. They had relatively small thyroid glands (clinically estimated <20gm) and normal serum levels of T_4. Although the serum TSH baseline levels of these women were considerably above normal (>10 μU/ml), their serum baseline PRL levels were normal (<30 mμg/ml). In comparison with the TSH responses of the normal women and men, the TSH responses of these women were greater at the 3 doses of TRH administered. Compared to the results of the men, 10, 25, and 800μg of TRH also released a greater amount of PRL in the women with the thyroid disorders; however, the PRL responses were not significantly different than those obtained in the normal women. The fact that the PRL responses were normal supports the clinical impression that they were euthyroid despite the elevated serum TSH levels. Previously, administration of TRH to women but not to men with hypothyroidism was found to elicit a greater than normal PRL as well as TSH response (14). Perhaps one reason why the TSH but not the PRL response is greater in women with thyroid disease is that the TRH induced TSH response is more easily inhibited by T_4 and T_3 than is the TRH-PRL response.

As recorded in Table 2, even 3 μg TRH elevates serum levels of both TSH and PRL in some women. Furthermore, TRH induced an essentially concomitant rise of both hormones (Table 3). TSH and PRL increased significantly at 5, 15, and 30 minutes but not at 2 minutes after a single intravenous injection of TRH.

Results recorded in Tables 4-8 once again emphasize the independence of the mechanisms involved in regulating PRL and TSH secretion. In women, PRL but not TSH rose to high levels during nursing (Table 4) while daily administration of 25μg of triiodothyronine (T_3) orally to 5 normal women inhibited the TSH but not the PRL response of TRH which was given iv once daily for 5 consecutive days (Table 5). As recorded in Table 6, the TSH and PRL-TRH responses of women and men with untreated thyrotoxicosis were virtually inhibited and when these same patients were treated and became euthyroid, the PRL responses were normal or higher than normal while the TSH response of 4 of the 7 women and 4 of the 5 men were still completely inhibited. Although the serum T_4 levels of subjects 5 and 11 were normal and they clinically appeared euthyroid, both the TSH and PRL responses of TRH of these subjects were greater than normal and, thus, they may have been in a very early stage of hypothyroidism. Results in Table 7 clearly demonstrate the increased TSH and PRL responses of TRH in subjects with hypothyroidism and the marked decrease in these responses during thyroxine therapy.

Table 2 EFFECT OF 3μg TRH

Women Subjects	TSH Minutes 0	+15	+30	Maximal Δ rise	PRL Minutes 0	+15	+30	Maximal Δ rise
	μU/ml				mμg/ml			
1	3.8	11.9	10.1	8.1	6.0	12	6.0	6.0
2	8.8	13.6	11.8	4.8	5.0	12	10.0	7.0
3	14.8	31.0	27.8	16.2	5.0	28	17.0	23.0
4	36.9	69.8	66.0	32.9	3.0	38	30.0	35.0
5	41.0	70.6	66.5	29.6	5.0	55	44.0	50.0
Mean	21.0	39.4	36.4	18.3	4.8	29	21.4	24.2

3μg TRH iv at 0 time.

Table 3 TIME OF TSH AND PRL RELEASE AFTER A SINGLE INTRAVENOUS INJECTION OF TRH

Subject	T4 μg%	Basal Level TSH	PRL	Minutes +2 TSH	PRL	+5 TSH	PRL	+15 TSH	PRL	+30 TSH	PRL
		maximal Δ μUTSH or mμgPRL/ml serum									
1	3.5	20	11.0	-2	-6.0	6	18	15	64	28	51
2	2.7	27	5.0	-1	1.0	5	4	26	19	34	36
3	6.5	40	8.0	1	0.0	33	34	46	87	33	52
4	3.3	10	3.0	6	10.0	28	48	71	93	102	93
5	5.4	44	10.0	-1	1.0	28	56	139	>90	169	>90
Mean	4.2	28	7.4	3	1.5	20	32	59	70	73	64

800μg TRH iv at 0 time to 5 euthyroid or mildly hypothyroid women.

Table 4 SERUM LEVELS OF PRL AND TSH IN WOMEN BEFORE AND DURING NURSING

Days Post Partum	Minutes of Nursing 0	30	60	Minutes of Nursing 0	30	60
	PRL mμg/ml			TSH μU/ml		
7	16	350	150	<2.5	<2.5	<2.5
19	8	190	170	<2.5	<2.5	<2.5
20	12	240	75	<2.5	<2.5	<2.5
35	8	120	120	<2.5	<2.5	<2.5
82	4	192	104	<2.5	<2.5	<2.5
83	3	220	151	<2.5	2.8	4.4
84	4	96	34	<2.5	<2.5	<2.5

Interestingly, even "physiological" doses of thyroxine completely inhibited the TSH response of 3 of the 4 subjects. As recorded in Table 8, even though the basal levels of PRL were elevated while subjects with schizophrenia were taking large doses of chlorpromazine, the PRL responses of TRH were greater than normal while the concomitant release of TSH was normal. Administration of L-DOPA to a patient with Parkinson's disorder for 12 months decreased both the TSH and PRL response of TRH but only the PRL response was decreased when another similar patient was treated with L-DOPA for 2 weeks.

Table 5 EFFECT OF TRIIODOTHYRONINE ON THE RESPONSE OF pGLU-HIS-PRO-NH_2 (TRH) IN FIVE NORMAL WOMEN

	TSH				PRL			
	Minutes				Minutes			
Day	0	15	30	Maximal Δ rise	0	15	30	Maximal Δ rise
	μU/ml				mμg/ml			
1	<2.5 ±0	32 ±16	37 ±17	34.5	9.4 ±5.9	52 ±9	46 ±14	42.6
2	<2.5 ±0	12 ±4.3	13 ±3.7	10.5	16 ±4.5	49 ±10	47 ±10	33.0
3	<2.5 ±0	6.0 ±1.9	7.0 ±2.7	4.5	29 ±10	55 ±10	45 ±12	26.0
4	<2.5 ±0	4.0 ±1.5	4.0 ±1.7	1.5	15 ±5	46 ±9	35 ±6	31.0
5	2.7 ±.3	3.4 ±.9	3.0 ±.5	.7	16 ±6	60 ±16	44 ±14	44.0

800μg TRH iv daily at 0 time. After first TRH injection, 25μg T_3 orally each day. Values are means (5) ±SE.

Table 6 TSH AND PRL RESPONSE OF TRH

	Thyrotoxic						Euthyroid					
	TSH			PRL			TSH			PRL		
	Minutes			Minutes			Minutes			Minutes		
Subject	0	+15	+30	0	+15	+30	0	+15	+30	0	+15	+30
	μU/ml			mμg/ml			μU/ml			mμg/ml		
	Women											
1	<2.5	<2.5	<2.5	6.0	10.0	9.0	<2.5	<2.5	<2.5	19.0	33.0	29.0
2	<2.5	<2.5	<2.5	7.0	11.0	9.0	<2.5	<2.5	<2.5	9.0	45.0	33.0
3	<2.5	<2.5	<2.5	8.0	14.0	15.0	<2.5	<2.5	<2.5	18.0	70.0	31.0
4	<2.5	<2.5	<2.5	15.0	15.0	16.0	<2.5	<2.5	<2.5	14.0	36.0	36.0
Mean	<2.5	<2.5	<2.5	9.0	13.0	12.0	<2.5	<2.5	<2.5	15.0	46.0	32.0
5	<2.5	<2.5	<2.5	12.0	20.0	15.0	26.0	123.0	175.0	14.0	100.0	100.0
6	<2.5	<2.5	<2.5	14.0	15.0	20.0	<2.5	17.0	18.0	15.0	100.0	75.0
7	<2.5	<2.5	<2.5	11.0	11.0	11.0	4.4	46.0	46.0	9.0	46.0	34.0
Mean	<2.5	<2.5	<2.5	12.0	15.0	15.0	11.0	62.0	80.0	13.0	82.0	70.0
	Men											
1	<2.5	<2.5	<2.5	7.0	6.0	6.0	<2.5	<2.5	<2.5	5.0	18.0	14.0
2	<2.5	<2.5	<2.5	6.0	17.0	13.0	<2.5	<2.5	<2.5	19.0	33.0	30.0
3	<2.5	<2.5	<2.5	5.0	11.0	7.0	<2.5	2.5	2.5	14.0	32.0	38.0
Mean	<2.5	<2.5	<2.5	6.0	11.0	7.0	<2.5	<2.5	<2.5	13.0	28.0	27.0
1	<2.5	<2.5	<2.5	8.0	12.0	9.0	9.0	51.0	59.0	9.0	80.0	100.0

800μg TRH iv at 0 time. While euthyroid, patients were on no therapy.

DISCUSSION: pGlu-His-ProNH_2, known to be in the hypothalamus of man (20,21), when administered as a single intravenous injection to normal women and men

Table 7 TSH AND PRL RESPONSE OF TRH

Women Subjects	Hypothyroid						Euthyroid					
	TSH			PRL			TSH			PRL		
	Minutes			Minutes			Minutes			Minutes		
	0	+15	+30	0	+15	+30	0	+15	+30	0	+15	+30
	μU/ml			mμg/ml			μU/ml			mμg/ml		
1	380	1661	1862	15	211	128	13.0	53.0	78.0	10	31	37
2	279	655	630	14	200	200	<2.5	<2.5	<2.5	15	35	26
3	185	210	264	8	155	152	<2.5	<2.5	<2.5	5	35	35
4	32	126	176	10	170	190	2.5	2.5	2.5	3	64	60
Mean	219	663	733	12	184	168	5.0	15.0	21.0	8	42	40

800μg iv at 0 time. While euthyroid, subject 1 was taking 200μg T_4 daily and the others 300μg.

Table 8 EFFECT OF CHLORPROMAZINE OR L-DOPA ON THE TRH RESPONSE IN EUTHYROID PATIENTS

Subject	Dose mg/day	Duration Months	TSH Minutes 0	TSH Minutes +15	TSH Minutes +30	TSH Max. Δ Rise	PRL Minutes 0	PRL Minutes +15	PRL Minutes +30	PRL Max. Δ Rise
			μU/ml				mμg/ml			
Chlorpromazine										
1	500	1.0	7.3	20.4	22	14.7	135.0	270	270	135.0
2	400	1.5	<2.5	31.0	40	37.5	49.0	228	160	179.0
3	200	3.0	5.0	23.0	32	25.0	95.0	165	175	80.0
4	350	1.5	<2.5	12.0	14	11.5	24.0	100	100	76.0
L-DOPA										
1	0	-	2.5	15.0	20	17.5	10.0	26	30	20.0
	3000	12.0	<2.5	5.0	10	8.5	5.0	7	11	6.0
2	0	-	<2.5	25.0	32	29.5	2.5	25	30	27.5
	1500	0.5	2.5	30.0	41	39.5	0.0	11	10	10.0

800μg TRH iv at 0 time.

invariably elevated serum levels of both TSH and PRL. Even 3 and 10μg of pGlu-His-ProNH_2 elevated PRL and TSH levels and a greater rise of PRL than TSH occurred at all three dosages, 10, 25, and 800μg of TRH administered. Both TSH and PRL rose at 5 but not at 2 minutes and their peak rise was at 15 or 30 minutes. Since minimally effective doses of pGlu-His-ProNH_2 release both TSH and PRL in man it is probable that endogenous TRH levels which stimulate TSH release also must stimulate PRL release. Furthermore, levels of thyroid hormone, an important determinant of the TSH response of TRH in animals and man, also influence the magnitude of the PRL response of TRH in man. As previously reported (14) and from the results in this study of the effect of TRH in the same patients while they were thyrotoxic or hypothyroid and again while euthyroid, it is apparent that physiological amounts of thyroid hormone definitely determine the amount of PRL as well as TSH that is released by TRH.

The possibility that the same hypothalamic releasing hormone, pGlu-His-ProNH_2,

participates in the physiological regulation of both TSH and PRL was most unexpected, especially since there are no a priori reasons relating their functions. The concept has been developed that for every pituitary hormone there is a specific companion hypothalamic hormone controlling its release. However, the critical evidence (Table 1-A and B) obtained in normal women and men shows that the minimal effective dose of TRH and the effective dose range of pGlu-His-ProNH$_2$ in releasing TSH and PRL are the same. These two results compel us to suggest that pGlu-His-ProNH$_2$ plays a dual role by participating in the regulation of both TSH and PRL secretion.

Though our results make it seem likely that pGlu-His-ProNH$_2$ participates in the regulation of PRL as well as TSH secretion under normal circumstances a number of alternative proposals might be offered. For example, pGlu-His-ProNH$_2$ may be so active in stimulating PRL release because it is closely related in chemical structure to the true hypothalamic prolactin releasing hormone. To investigate this possibility 30 TRH analogs or derivatives were administered to monkeys (17). Many of the analogs are active in releasing TSH in mice (22) as well, but as yet none have been found which are more potent than TRH in releasing PRL in monkeys. The most active analogs in releasing TSH in mice were also the most active analogs in releasing PRL in monkeys. Two hypothalamic PRH's are possible but theoretically this seems unlikely. Delivery of pGlu-His-ProNH$_2$ via specific portal vessels only to the TSH cells but not the PRL cells of the pituitary is another alternative that would prevent PRL from being released by pGlu-His-ProNH$_2$; however, a selective and specific micro circulation of this kind has not been demonstrated in the pituitary.

A third possibility is that TRH does not act directly on the pituitary to release PRL, especially since this has been difficult to demonstrate *in vitro*, but rather on the hypothalamus to inhibit PIF directly or indirectly and in this way elevates PRL. Against this possibility is our findings that TRH administered to men and women with high PRL levels while taking chlorpromazine have an even greater than normal PRL response to TRH while the TSH response was normal (23). Presumably, in these patients PIF secretion is inhibited and, thus, it seems unlikely that TRH acts by inhibiting PIF secretion. The results of the chlorpromazine studies, the L-DOPA studies (23) on the inhibition of the TRH-PRL response (also the TSH response 23,24) in men and women, as well as the inhibitory effect of partially purified porcine PIF on the TRH-induced release of PRL from the monkey pituitary *in vitro* (unpublished) all suggest that PIF inhibits the TRH induced release of PRL.

Although a single hormone, TRH, probably influences both TSH and PRL secretion, despite the fact that under some circumstances each hormone can be secreted independently. Serum PRL but not TSH increases in animals and man after section of

the pituitary stalk or after administration of chlorpromazine (8, 25). During breast feeding women have high serum levels of PRL but not TSH (Table 4). Presumably, in the examples mentioned, PRL increases as a result of the decreased secretion of PIF (PIH) from the hypothalamus.

Since pGlu-His-ProNH$_2$ releases more PRL than TSH in man as well as in monkeys (17) it could be considered that this tripeptide hormone is really not TRH but only chemically related. Perhaps, the graded quantitative interaction (26) between the thyroxine (T_4), triiodothyronine (T_3) and pGlu-His-ProNH$_2$ involved in the secretion of TSH is one of the most important reasons for concluding pGlu-His-ProNH$_2$ is indeed the physiological hypothalamic releasing hormone for TSH. A specific inter-relatedness between the thyroid hormones and the amount of prolactin released by TRH is demonstrated by the results of our studies. However, this seems to be demonstrable only when T_3 or T_4 are deficient or in excess amounts and, thus, in comparison to the effect on TSH the thyroid hormones may be more of a secondary rather than a primary regulator of PRL secretion.

It will be of fundamental importance to determine the biological reasons why this single hypothalamic hormone regulates both pituitary hormones. Therefore we feel the proposed term PTRH rather than TRH more accurately indicates the full spectrum of biological activities of pGlu-His-ProNH$_2$ in man.

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