PROLACTIN AND THYROTROPIN RELEASE IN MAN BY SYNTHETIC PYROGLUTAMYL-HISTIDYL-PROLINAMIDE

by

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SUMMARY Synthetic pyroglutamyl-histidyl-prolinamide or thyrotropin-releasing-hormone (TRH) stimulates secretion of prolactin (PRL) as well as thyrotropin (TSH) but has no consistent effect on serum levels of growth hormone (GH), luteinizing hormone (LH) or follicle stimulating hormone (FSH) in man. After intravenous injection of as little as 10 μg TRH, both serum PRL and TSH levels rise rapidly. The PRL and TSH response was greater in normal women than in normal men and both responses were greatly enhanced in hypothyroid patients and virtually abolished in hyperthyroid patients. The possibility that p-Glu-His-Pro-NH_2 is also a "natural physiological" regulator of PRL in man is evident.

INTRODUCTION A great deal is known about the factors which regulate prolactin (PRL) secretion in animals (1) but the control mechanisms which influence PRL secretion in man remain largely unknown (2). This uncertainty derives from the inability, until recently, to isolate human PRL as a separate pituitary hormone free of human GH.

With the development of a specific radioimmunoassay for human prolactin (3) the unequivocal identification of a separate human PRL was achieved. It was recognized that an immunological relationship existed between primate and non-primate PRL (4,5) and that it was possible to purify human and monkey PRL by the application of affinity chromatography using specific antibodies to human placental lactogen (6). More recently the separation of human PRL and HGH has also been achieved by electrophoretic techniques (7,8).

Most evidence indicates that in animals (1) PRL secretion is primarily controlled by tonic inhibition via a hypothalamic factor called prolactin inhibiting factor (PIF). Clinical observations in man also provide evidence for a similar control mechanism, as we have observed immediate and sustained serum prolactin elevations following stalk section of the pituitary or in the presence of hypothalamic, but not pituitary lesions (unpublished observations). Presumably the increased prolactin concentration in this circumstance occurs as a consequence of the absence of PIF.

Evidence that the hypothalamus may also secrete a prolactin releasing factor (PRF) was presented by Nicoll and co-workers in 1969 (9) and by Krulich et al (10) and Valverde-R and Chieffo (11). Direct evidence that TRH might be implicated in prolactin release was provided by the studies of Tashjian et al (12) who reported that the addition of TRH stimulated the production of prolactin in vitro in 2 clonal strains of rat pituitary cells. In the present study we report that in man, a single intravenous injection of as little as 10 to 25 μg TRH produces a remarkable increase in serum PRL as well as TSH.

METHODS TRH was synthesized as previously described (13) and all hormones were measured in duplicate by radioimmunoassay methods (3,14). TRH dissolved in 1 ml normal saline was injected intravenously within 15 to 20 seconds into ambulatory patients after collection of the control blood sample.

RESULTS As recorded in Table I, TSH and PRL rose 15 and 30 minutes after 25 or 800 μg TRH in all of the 6 normal women and men. Both TSH and PRL responses were greater in the women.

The TSH and PRL responses obtained after repeated single intravenous injections of increasing doses of TRH to two normal women are recorded in Table II. For both subjects, 3 and 10 μg of TRH were administered at 3 hour intervals on the same day. Three months later, these women received 25, 50, 100 and 200 μg TRH at 2 hour intervals and 12 hours later, on the subsequent

TABLE I. RELEASE OF TSH AND PRL BY pGLU-HIS-PRO-NH_2* IN NORMAL WOMEN AND MEN

	WOMEN											
	25μg TRH						800μg TRH					
	minutes						minutes					
Subject	0	15	30	0	15	30	0	15	30	0	15	30
	μU TSH/ml			mμg PRL/ml			μU TSH/ml			mμg PRL/ml		
1	5	25	25	10	28	15	4	42	34	5	>120	50
2	7	46	38	45	73	64	5	90	93	24	78	67
3	4	27	22	14	51	35	4	25	28	14	43	33
4	<2.5	15	15	24	>120	>120	<2.5	21	21	11	>100	>120
5	3	21	18	13	110	90	3	83	95	13	>105	55
6	3	19	16	11	34	29	3	18	21	13	34	27
Mean	4	26	22	20	69	59	4	47	49	13	80	59
	MEN											
1	<2.5	11	9	0	14	14	3	6	7	0	14	12
2	3	10	12	12	14	15	7	23	23	10	20	19
3	<2.5	10	8	12	46	31	<2.5	15	18	12	60	47
4	3	25	29	12	40	22	3	20	31	9	31	25
5	3	12	11	13	31	25	<2.5	15	14	12	50	42
6	<2.5	9	6	11	21	18	<2.5	5	6	12	25	26
Mean	3	13	13	10	28	21	3	14	17	9	33	29

*25μg or 800μg TRH injected iv at 0 time.

TABLE II. EFFECT OF REPEATED ADMINISTRATION OF pGLU-HIS-PRO-NH_2* IN TWO NORMAL WOMEN

	Subject G.A.R.										Subject C.W.									
	minutes										minutes									
TRH	0	15	30	0	15	30	0	15	30	TRH	0	15	30	0	15	30	0	15	30	
μg	μU TSH/ml			mμg PRL/ml			mμg GH/ml			μg	μU TSH/ml			mμg PRL/ml			mμg GH/ml			
3	4	10	9	5	13	12	-	-	-	3	3	7	5	12	13	14	-	-	-	
10	5	21	19	20	43	29	-	-	-	10	2	8	6	13	18	14	-	-	-	
25	4	21	22	14	51	35	2	1	1	25	3	15	10	-	-	-	1	2	2	
50	7	37	26	10	47	27	1	2	2	50	4	24	21	11	40	26	8	11	12	
100	7	33	30	12	34	24	2	2	2	100	7	23	22	6	30	25	2	2	3	
200	7	32	30	12	35	24	2	3	2	200	5	18	22	15	24	27	11	11	8	
400	3	21	21	13	48	26	2	3	4	400	3	6	8	12	24	15	3	2	3	
800	4	17	18	10	10	18	2	3	2	800	3	10	11	15	27	22	1	3	4	
800_1	4	28	29	14	43	33	-	-	-	800_1	6	23	26	-	-	-	-	-	-	

*Various doses of TRH injected iv at 0 time. 1-TRH injected at another time 3 months later.

day, 400 and 800 μg TRH was administered at a 3 hour interval. The last recorded responses to 800 μg TRH were obtained at another time 3 months later. PRL and TSH rose after injection of 10 μg of TRH. In subject G.A.R., a slight increase occurred even after injection of 3 μg TRH. The largest TSH and PRL responses occurred after injection of 50 and 25 μg of TRH, respective-

ly in subject G.A.R. and 50 μg in subject C.W. Subsequently, after injection of larger doses of TRH, the TSH as well as the PRL responses were less. In all studies, GH levels did not change after injection of TRH.

TABLE III. RELEASE OF TSH AND PRL BY SYNTHETIC pGLU-HIS-PRO-NH_2* IN EUTHYROID WOMEN WITH NON-TOXIC GOITERS

	Decreased TRH-TSH Response							Normal TRH-TSH Response						
	minutes							minutes						
Subject	0	15	30	0	15	30	Subject	0	15	30	0	15	30
	μU TSH/ml			mμg PRL/ml				μU TSH/ml			mμg PRL/ml		
1	4	11	12	3	60	53	1	7	24	24	14	37	26
2	5	13	16	8	143	173	2	10	23	27	0	26	21
3	6	15	15	22	37	20	3	6	24	33	6	50	40
4	3	8	11	7	24	24	4	3	25	29	8	30	24
5	5	8	8	9	36	35	5	5	19	20	0	20	22
Mean	5	11	12	10	60	61		6	23	27	6	33	27

*800 μg TRH injected iv at 0 time

As recorded in Table III, serum PRL levels rose after 800 μg TRH in each of the ten euthyroid women with non-toxic goiters. These patients were 22-73 years of age, serum thyroxine levels ranged 4.5-9.1 μg% (normal 4-10) and goiter size 40-120 gm (normal <25 gm). The mean increase in serum PRL concentration between 0 and 15 or 30 minutes (Δmμg/ml) calculated from the results of these ten patients, were 50 and 51 respectively and the mean PRL concentration at 0 minutes was 10 mμg/ml. Control serum PRL levels of normal subjects range from 0 to 30 mμg/ml. PRL increased maximally 15 minutes and TSH 30 minutes after the administration of TRH. The greatest PRL response occurred in a woman with a decreased TSH response. Omitting this latter re-

TABLE IV. EFFECT OF pGLU-HIS-PRO-NH_2* ON SERUM LEVELS OF TSH, PRL, LH AND FSH IN WOMEN WITH UNTREATED THYROTOXICOSIS

	minutes											
Subject	0	15	30	0	15	30	0	15	30	0	15	30
	μU TSH/ml			mμg PRL/ml			mIU LH/ml			mIU FSH/ml		
1	<2.5	<2.5	<2.5	21	23	23	23	38	43	3	4	3
2	3.8	3.4	2.9	12	5	7	13	16	15	8	8	9
3	<2.5	<2.5	<2.5	9	15	13	2	3	3	<1	<1	<1
4	3.1	2.7	3.0	6	7	6	23	20	22	14	15	13
5	<2.5	<2.5	<2.5	7	17	14	28	32	35	9	9	13
6	<2.5	<2.5	<2.5	15	25	24	33	33	34	13	14	20
Mean	2.8	2.6	2.7	12	15	15	20	24	25	8	9	10

*800 μg TRH injected iv at 0 time.

sponse, the PRL responses were essentially the same in the two groups, even though the magnitude of the TSH responses were different.

Results in Table IV show that 800 μg of TRH had little or no effect on serum levels of TSH, PRL, LH or FSH in 6 women with untreated toxic goiters. The range of ages, serum thyroxine levels and goiter size were 23-46 years, 10.2-19.5 μg% and 50-200 gm, respectively. The mean increase of PRL (Δmμg/ml) at 15 and 30 minutes was 3. In one subject, LH concentration increased, but in the other 5 women there was no change. TSH and FSH levels remained essentially unchanged.

The TSH and PRL responses which were obtained in 9 women with various types of thyroid disorders are recorded in Table V. The control TSH level at 0 time of all the women was elevated (>10 μU/ml). The first five euthyroid

TABLE V. RELEASE OF TSH AND PRL BY pGLU-HIS-PRO-NH_2* IN WOMEN WITH THYROID DISORDERS

	25 μg TRH						800 μg TRH					
	minutes						minutes					
Subject	0	15	30	0	15	30	0	15	30	0	15	30
	μU TSH/ml			mμg PRL/ml			μU TSH/ml			mμg PRL/ml		
1	28	146	148	0	54	46	27	160	180	3	47	40
2	19	94	79	-	61	45	26	100	140	2	45	-
3	118	>250	>250	15	110	90	100	246	307	-	155	152
4	-	-	-	-	-	-	81	172	173	3	72	69
5	95	250	238	12	55	59	54	230	272	5	72	66
6	10	29	30	0	43	16	13	53	69	4	44	39
7	-	-	-	-	-	-	65	120	126	6	53	39
8	17	97	91	2	45	33	14	159	183	3	65	56
9	11	68	62	28	77	57	18	191	186	5	41	65
Mean	43	133	128	10	64	49	44	159	181	4	66	66

*25 or 800 μg TRH injected iv at 0 time.

or slightly hypothyroid subjects had thyrotoxicosis which had previously been treated and the last 4 subjects had non-toxic goiters. Ranges of ages, serum thyroxine levels, and goiter size were 28-48 years, 3.0-7.3 μg% and 20-128 gm respectively. Seven women received 25 and 800 μg of TRH at 2 to 4 month intervals. A greater than normal TSH rise occurred with both doses of TRH. Previously, it has been found that the mean TSH rise (ΔμU/ml) of normal women 30 minutes after 25 μg TRH is 10 and after 800 μg is 25. The PRL responses at 15 minutes were slightly greater than at 30 minutes and the mean (Δmμg/ml)

PRL rise at 15 and 30 minutes after 25 μg TRH was 54 and 39 and after 800 μg TRH was 63 and 61.

Results of 5 patients with untreated myxedema are recorded in Table VI.

TABLE VI. RELEASE OF TSH AND PRL BY pGLU-HIS-PRO-NH_2* IN PATIENTS WITH UNTREATED MYXEDEMA

Subject	T_4 μg%	TRH μg		minutes -15	0	15	30	45	60	75	120
L.P.	3.0	0_1	GH	5	4	14	17	9	6	4	4
			PRL	16	9	13	8	16	17	13	15
L.P.	2.5	800	TSH	-	580	2120	2800	-	5360	-	-
			PRL	-	22	383	315	-	-	-	-
			GH	-	<1	1	1	-	-	-	-
			LH	-	33	10	14	-	-	-	-
			FSH	-	5	6	-	-	-	-	-
M.M.	2.0	800	TSH	-	170	222	310	-	-	-	-
			PRL	-	8	67	65	-	-	-	-
$M.M._2$	3.0	800	TSH	-	<2.5	3	5	-	5	-	-
			PRL	-	10	31	37	-	23	-	-
M.P.	2.0	800	TSH	-	127	210	220	-	-	-	-
			PRL	-	16	>120	>120	-	-	-	-
G.H.	2.0	800	TSH	135	115	165	280	340	310	295	220
			PRL	-	8	30	33	22	19	-	-
			LH	13	11	11	11	9	8	8	7
			FSH	6	7	6	5	7	9	9	9
A.T.	1.5	800	TSH	-	221	366	420	-	600	-	-
			PRL	-	9	24	27	-	21	-	-

*TRH was injected iv at 0 time. Values are recorded per ml serum as: μU TSH, mμg PRL and GH, mIU LH and FSH. 1-Insulin Tolerance Test. 2-On 200μg T_4 daily.

L.P., M.M., and M.P. were women and G.H. and A.T. men. Especially noteworthy is the marked TSH and PRL responses without a concomitant change in the GH, LH or FSH levels of subject L.P. Furthermore, during an insulin tolerance test in this subject, GH, but not PRL increased. TSH and PRL also increased in the other 4 subjects but to a smaller extent. Like TSH, the PRL response may be greater in women than men (14).

Sequential studies of TRH in a 53 year old woman with myxedema and an enlarged sella turcica, are summarized in Table VII. Before treatment, TRH induced a marked rise of TSH and PRL without a concomitant increase of LH or FSH while glucagon had no effect on levels of GH or PRL. Administration of 50 μg thyroxine daily decreased the TSH response but had no effect on the PRL

TABLE VII. EFFECT OF THYROXINE ON RELEASE OF TSH AND PRL BY pGLU-HIS-PRO-NH_2* IN A 53 YEAR OLD WOMAN WITH AN ENLARGED SELLA TURCICA AND AN ELEVATED SERUM TSH LEVEL

PBI	T_4	T_4 R	TRH		minutes							
µg%	µg%	µg	µg		0	15	30	40	60	80	120	240
		0	0_1	GH	<1	-	-	<1	-	<1	1	2
				PRL	19	-	-	19	-	-	17	21
				TSH	347	-	-	232	-	242	228	262
2.3	0.7	0	800	TSH	380	1661	1862	-	2287	-	-	-
				PRL	15	221	-	-	128	-	-	-
				LH	25	18	-	-	15	-	-	-
				FSH	31	31	-	-	-	-	-	-
		50_2	800	TSH	105	515	775	-	-	-	-	-
				PRL	5	176	184	-	-	-	-	-
		50_3	800	TSH	90	590	>1000	-	588	-	-	-
				PRL	8	292	207	-	190	-	-	-
5.1	5.7	$50\text{-}100_4$	800	TSH	20	180	240	-	270	-	-	-
				PRL	2	36	53	-	42	-	-	-
				LH	10	13	17	-	9	-	-	-
				FSH	59	63	63	-	67	-	-	-
		200_5	800	TSH	13	53	78	-	70	-	-	-
				PRL	10	31	37	-	33	-	-	-

*TRH was injected iv at 0 time. Values are recorded per ml serum as: µU TSH, mµg PRL and GH, mIU LH and FSH. 1-1 mg Glucagon was injected sc at 0 time; 2-T_4 was administered for 1 month; 3-T_4 was administered for 7 weeks; 4-50µg T_4 was administered for 2.5 months then 100µg T_4 for 1 month; 5-200µg T_4 was administered for 1 month.

response. During treatment with 100 µg thyroxine both the TSH and PRL responses were markedly decreased.

DISCUSSION Unequivocal evidence has been obtained that p-Glu-His-Pro-NH_2 (TRH), known to be in the hypothalamus of man (15,16), increases serum PRL levels in man. TRH stimulated a marked increase of serum PRL and TSH without a concomitant rise in GH, LH or FSH. The increase in TSH and PRL was rapid following the intravenous injection of TRH. The marked PRL rise induced by TRH but not insulin hypoglycemia in the same patient also indicates the specificity of the TRH response. These results once again demonstrate the specificity of the radioimmunoassay for human PRL and, indeed, support the findings that PRL exists in the human pituitary.

Since TRH acts on the pituitary to release TSH, it seems likely that TRH acts at this site as well to release PRL in man. Tashjian's studies (12) of the effect of TRH on the rat pituitary *in vitro* support this conclusion.

Perhaps the greater PRL and TSH responses in women as compared to men

are secondary to the differences in circulating estrogen levels. We have also observed this sex difference in PRL concentrations in subjects who are on phenothiazines or who have chronic renal failure: women have much greater elevations of serum prolactin than men.

There appears to be a definite relationship between the degree of function of the thyroid and the magnitude of the TRH stimulated PRL release. Responses in women with hyperthyroidism were small and those with hypothyroidism were large. Sequential studies in one patient indeed show that the magnitude of the PRL response to TRH is influenced by the circulating thyroxine level. However, as recorded in Table I, the magnitude of the TSH and PRL responses to TRH did not parallel one another.

It appears that there is a much greater depletion of pituitary PRL than of TSH. The content of PRL and TSH in the human pituitary is approximately 100 μg (17) and 1000 mU or 25 μg (18) respectively. With an immediate total depletion of this store the serum PRL and TSH concentration would increase to 10 mμg/ml and 100 μU/ml respectively (assuming the same volume of distribution of 10 liters for both hormones). The $T^1/2$ of TSH and PRL is approximately 60 and 20 minutes respectively. Hence, we must postulate that TRH enhances PRL synthesis as well as release in order to explain the magnitude of some of the responses where the increase in PRL is more than 200 ng/ml in 15 minutes and remains elevated for at least 30 minutes. While many times more than the total pituitary prolactin store must be secreted to obtain the increase in PRL observed following TRH, only 20% of TSH would be required to be released to raise serum TSH to the levels observed.

PRL levels were not elevated in the control serum samples of untreated hypothyroid patients who had elevated TSH levels. These results suggest that TRH secretion is not elevated in untreated hypothyroidism and that the increased TSH secretion must be due to an increased sensitivity of the pituitary to TRH. A similar mechanism may explain the galactorrhea and elevated prolactin concentration which has been found in some hypothyroid subjects (19).

These studies raise important questions about the hypothalamic regulation of PRL secretion in man. They indicate that the effect of TRH may be even greater on the release of PRL than on TSH suggesting that perhaps TRH may be an important physiological regulator of PRL secretion in man. Alternatively, hypothalamic PRH may be related chemically to TRH, hence the overlapping function; or TRH may have other biological functions than to regulate TSH secretion. If so the term TRH may not reflect the full spectrum of TRH actions.

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