Synthetic Thyrotropin-Releasing Hormone. Activity in Men and Women, Specificity of Action, Inhibition by Triiodothyronine, and Activity Orally†

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In this study normal women were more sensitive to synthetic TRH than normal men although their responses were more variable. TRH mainly stimulated release of TSH but there were exceptions since an increased release of other pituitary hormones occasionally occurred. Pretreatment with T_3 partially or completely inhibited the TRH response. TRH was active orally and produced prolonged elevation of serum TSH levels.

Chemical and biological data are in agreement that pGlu-His-Pro-NH_2 is the structure of porcine[1-6] and ovine[7-9] TRH. Recent evidence indicates also that this is probably the structure of bovine and human TRH.[10,11] Synthetic TRH releases TSH from the pituitary gland of normal men.[12-15] Highly purified natural TRH obtained from the hypothalami of pigs also was found to release TSH in human subjects.[16] The effectiveness in the human of the synthetic hormone of established organic structure as well as the evidence that this hormone is present in the hypothalamus of humans may be considered as one of the ultimate criteria which validates the general concept of neurohumoral control of the anterior pituitary gland in man. The time-response patterns of serum TSH levels after iv administration of partially purified porcine TRH and synthetic TRH are recorded in Figure 1.

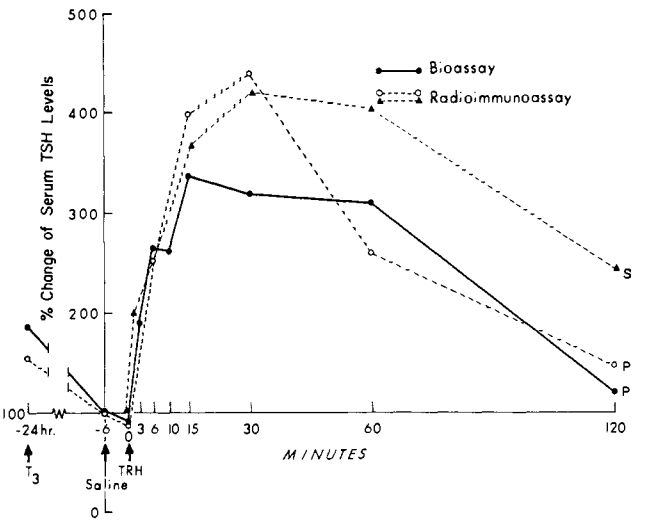

Figure 1.—Effect of porcine TRH (P) and synthetic TRH (S) on serum levels of TSH; 300 μg of partially purified porcine TRH was administered iv to a cretin pretreated with a single 25-μg dose of T_3 and 800 μg of synthetic TRH was administered iv to a normal adult male.

Now described are additional results from our continuing investigation of the activity and specificity of synthetic TRH in man as well as the differences in sensitivity of normal men and women to TRH, the effect of triiodothyronine (T_3) on the TRH response, and the activity of TRH when administered orally or by sequential intravenous injection.

Experimental Section

The clinical studies were performed at the Instituto Nacional de Nutricion, Mexico, D.F., Mexico. The subjects were 15 normal men and 13 normal women volunteers whose ages ranged from 19 to 46 years and weighed 50 to 72 kg.

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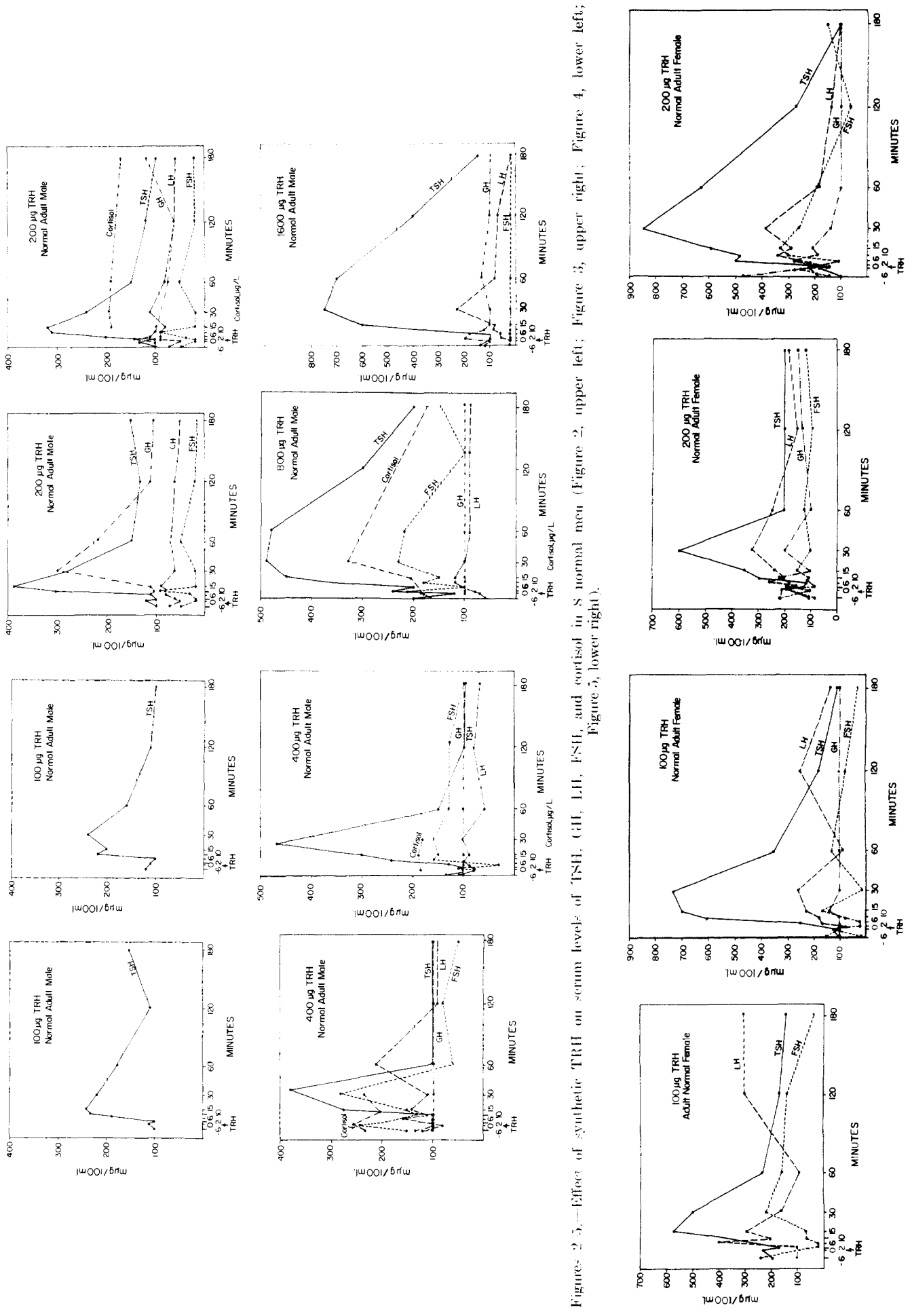
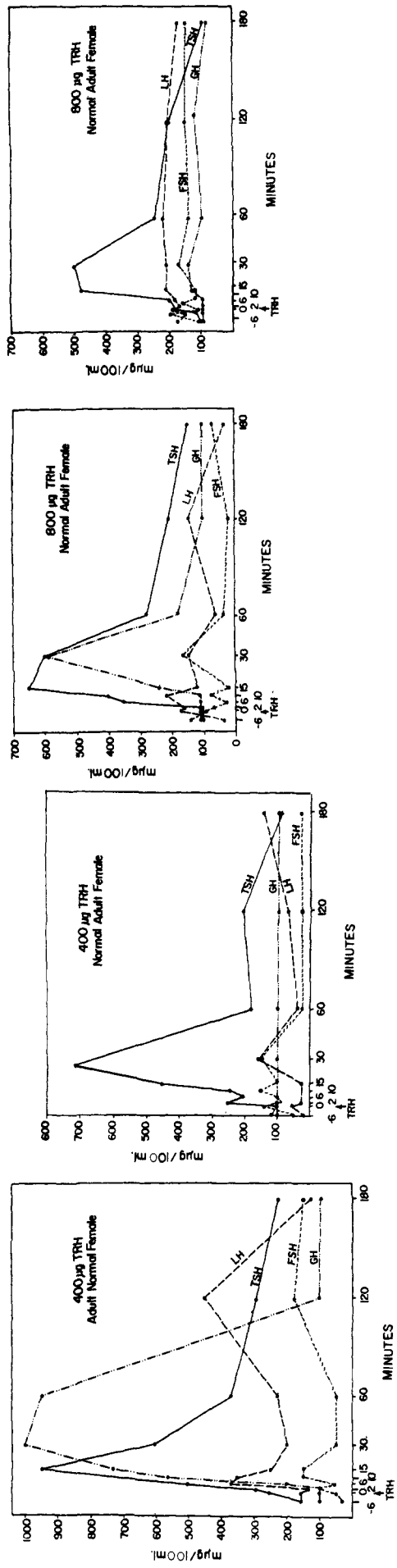


Figure 2-5.—Effect of synthetic TRH on serum levels of TSH, GH, LH, FSH, and cortisol in 5 normal men (Figure 2, upper left; Figure 3, upper right; Figure 4, lower left; Figure 5, lower right).



Figures 6-9.—Effect of synthetic TRH on serum levels of TSH, GH, LH, and FSH in 8 normal women (Figure 6, upper left; Figure 7, upper right; Figure 8, lower left; Figure 9, lower right.

The synthetic TRH was prepared by the method described in the companion paper by Chang, *et al.*[17] It was dissolved in normal saline, passed through a Millipore filter, and stored in sterile vials. Serum levels of TSH, LH, FSH, and GH were measured in duplicate by the double antibody pptn method described by Schalch, *et al.*[18], and Odell, *et al.*[19] Cortisol was measured by fluorometry.[20]

Subjects fasted overnight. During the tests, 3 subjects were lying down while the others were sitting upright. Some tests started at 8:30 AM and the others at 2:00 PM. During the control period, blood samples were taken at minus 6 min and then 1 ml of normal saline was injected iv and the second blood samples were taken at zero time. TRH in dosages ranging from 25 to 1600 µg in 1 ml of normal saline was then injected iv into the antecubital vein. Seven subjects had received T_3 (Cytomel) before injections of saline and TRH. Two men received 20 mg of TRH orally.

Results reported in equiv wt of the following human pituitary reference preparations: GH, 2 IU/mg; LH, 10 IU/mg, and FSH, 4 IU/mg, 2nd IRP-HMG and TSH, 5.7 IU/mg. The TSH IU are expressed in terms of the human pituitary standard A obtained from the National Institute of Medical Research, Mill Hill, London, England.

Results

Serum levels of TSH, GH, LH, FSH, and cortisol before and after injection of synthetic TRH in 8 men are recorded in Figures 2–5 and in 8 women are recorded in Figures 6–9. TSH rose significantly after the iv injection of TRH in each subject. Usually the rise of TSH was not detected for 6 min. The peak TSH rise occurred usually at 30 min and only occasionally at 15 min. As the dose of TRH was increased, greater rises in TSH levels were observed in men, 1600 µg eliciting the largest response. Although the TRH response of women was more variable, the response to 100 or 200 µg was much greater in women than in men. Levels of TSH returned to normal 2–3 hr after the injection of TRH.

LH, FSH, or cortisol levels of some patients rose only slightly, however, the rise in GH was greater. These rises coincided fairly well with the rise of TSH. FSH and/or LH levels rose slightly in 3 of the 8 women and in 1 of 6 men. GH rose in 2 of the 8 women and 1 of the 6 men. Cortisol, measured in only the men, rose slightly in 1 of 4 men.

Data in Figure 10 show that the oral administration of 100 µg of T_3 5–6 hr before injection of TRH to 4 men

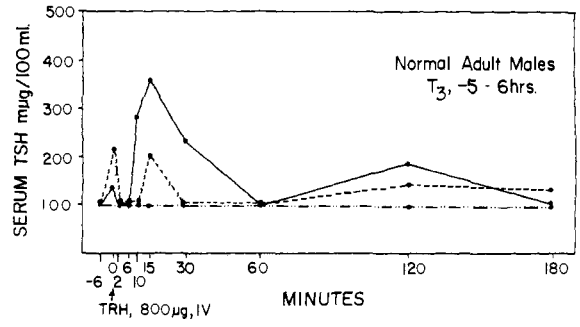


Figure 10.—Effect of T_3 on the response of synthetic TRH in 3 normal men.

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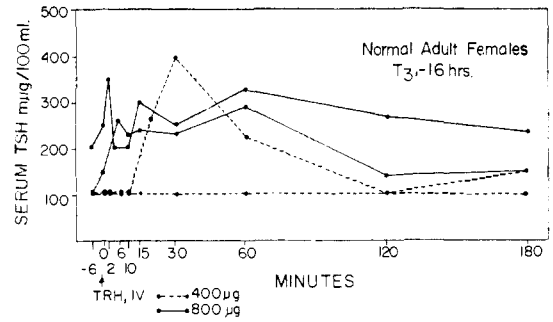

Figure 11.—Effect of T_3 on the response of synthetic TRH in 4 normal women.

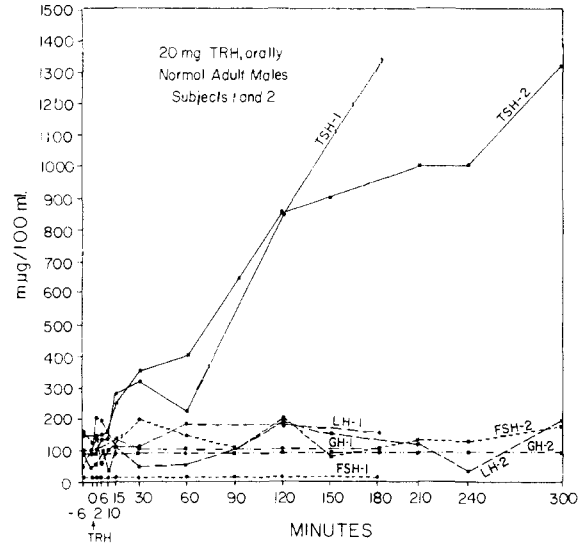

Figure 12.—Response of synthetic TRH after oral administration in 2 normal men.

completely or partially inhibited the TRH response in 3 subjects. There was little effect of T_3 in the fourth subject even though he was given 400 rather than 800 μg of TRH. Four women (Figure 11) also received 100 μg of T_3 orally but it was given 16 hr before the injection of 800 μg of TRH; their TSH rise also was partially or completely inhibited.

The greatest elevation in serum TSH levels occurred after oral administration of 20 mg of synthetic TRH to 2 men (Figure 12). TSH levels were measured over a 3-hr period in subject 1, and 5 hr in subject 2. In each instance, the levels were still rising at 3 and 5 hr. The first detectable TSH rise occurred 20 to 30 min after ingestion of TRH. No changes occurred in the serum levels of GH, FSH, and LH.

Results in Table I indicate that the administration of 25 μg of TRH iv to 2 adult normal females and 1 adult normal male significantly elevated serum TSH levels in each subject 15 and 30 min after injection of TRH. Furthermore, when 50, 100, and 200 μg of TRH was administered at 2.5-hr intervals on the same day, the per cent change in TSH levels in the female and male subjects was not greater than the responses obtained with 25 μg. To the female subjects on the subsequent day (12 hr after the last TRH injection), 400 and 800 μg of TRH was administered at 4-hr intervals. These latter responses were less or equal to the responses obtained when 25 μg of TRH was administered.

TABLE I

EFFECT OF SEQUENTIAL INJECTIONS OF TRH ON SERUM TSH LEVELS[a]

		TSH determinations						
	Dose of TRH,	μIU/ml Time in min			ΔμIU/ml Time in min		% change Time in min	
Subject	μg	0	+15	+30	+15	+30	+15	+30
Female 1	25	2.50	14.60	10.33	12.10	7.83	484	313
	50	3.75	24.00	20.66	20.25	16.50	540	440
	100	6.53	22.91	22.30	16.41	15.83	252	243
	200	4.66	18.00	21.50	13.40	17.84	287	382
	400	2.50	6.41	7.75	3.91	5.25	156	210
	800	2.60	9.58	10.58	6.98	7.98	268	306
Female 2	25	3.58	27.00	21.75	23.40	18.17	654	507
	50	7.41	36.50	26.41	29.09	19.00	392	256
	100	7.00	33.33	29.50	26.33	22.50	376	321
	200	7.16	32.41	30.00	25.25	22.84	352	318
	400	2.5	20.83	21.33	18.33	18.83	733	753
	800	3.5	17.41	18.08	13.91	14.58	397	416
Male 1	25	4.83	17.91	18.75	13.08	13.92	270	288
	50	10.5	26.91	30.16	16.41	19.60	156	186
	100	9.75	32.00	45.83	22.25	35.08	228	359

[a] After collection of blood at 0 time (at 8 AM) on the first day, 25 μg of TRH was injected iv and blood was collected again at 15 and 30 min. At 2.5-hr intervals, this testing sequence was repeated; the female subjects received 400 and 800 μg of TRH on the second day. TSH was measured by the NIH ^{131}I method and results are expressed in terms of μU of the human pituitary research standard A preparations obtained from the National Institute for Medical Research, Mill Hill, London, England.

Clinically, no adverse effects were noted in any of the subjects; blood pressure, pulse, and respiration remained unchanged. Most but not all patients have a bitter or salty taste in their mouth about 30 sec after the TRH injection which lasts only a few sec. Nausea occurred in less than 5% of the subjects about 30 sec after injection of TRH and lasted 60–90 sec. Also unchanged were results of the cbc, liver function tests, serum levels of cholesterol, total lipid, glucose, uric acid, and urea N measured 3 hr after injection of TRH.

Discussion

Synthetic TRH has been found to be unequivocally active in normal men and women and caused no adverse side effects. Interestingly, the responses to TRH were greater in women than men; however, the responses of women were more variable. Since the TRH responses were more variable in women, it will be necessary to study carefully and methodically what factor(s) may account for their varying sensitivity to TRH. Of great interest will be the determination of whether or not the greater response to TRH can in any way be related to the greater incidence of nontoxic goiters in women.

When serum TSH levels were measured as the index of the TRH response, the peak response occurred 15 or 30 min after quick iv administration; 25 μg of TRH significantly elevated serum TSH levels. When increasing amounts of TRH were sequentially administered on the same day to the same subject an increasingly greater TSH response was not observed. This may indicate an alteration in the sensitivity of the pituitary due to a release of thyrosine and triiodothyronine by the elevated TSH levels. A variety of studies will be necessary to understand these relationships.

In most instances, TRH stimulated only release of TSH. There were some notable exceptions in which the serum levels of 1 or 2 of the other hormones measured rose coincidently with the TSH rise. The latter changes were neither related to the dose of TRH nor the magnitude of the TSH response. Further studies will be necessary to elucidate the possible importance of the secretion of more than one pituitary hormone in response to TRH.

As has been found in animal studies,[5] whether or not T_3 inhibits the response to TRH will depend probably on its dose and time of administration as well as the dose of TRH. It will be of interest to determine what factor(s) may alter the sensitivity of this inhibitory effect of T_3.

TRH is indeed active when given orally to man[5,21] as was also found in mice.[5] The high sustained rise in the TSH level stimulated by oral administration of TRH indicates that this may be the method of choice in producing a prolonged effect. The relative absence in changes of the serum levels of the other hormones again shows the specificity of the TRH effect.

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Syntheses of Pyroglutamylhistidylprolinamide and Unusual Mass Fragmentation†

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The thyrotropin-releasing hormone, which is pGlu-His-Pro-NH_2, has been synthesized by a new procedure. This synthesis has also served particularly as a general procedure for the synthesis of many analogs of TRH to study structure-activity relationships. TRH was also synthesized by a solid phase method which couples a dipeptide, pyroglutamylhistidine, to a proline-resin. Low- and high-resolution mass spectra show that the fragmentation pattern of TRH is more specific to the histidine characteristics of this tripeptide than it is to the behavior of peptides in general.

Two syntheses of the porcine thyrotropin-releasing hormone (TRH), which is pGlu-His-Pro-NH_2 (**1**), and citations to pertinent publications of other investigators on the synthesis of TRH are detailed in companion papers.[1,2] These several syntheses of pGlu-His-Pro-NH_2 were a part of, or stemmed from, the structural elucidation of both porcine and ovine TRH. The TRH of all mammalian species[3] which have been reported upon to date has the same structure (**1**).

We have now synthesized TRH by a new procedure, which has been effective not only in making available larger amounts of the pure hormone, but the key steps have very effectively served for synthesis of many structural analogs of TRH.[4] This synthesis (of general utility for TRH and related tripeptides) has the advantage of providing two intermediates, **5** and **6**, which are generally crystalline and provide intermediate purifications. These advantages became increasingly apparent as many of the analogs were being synthesized.[4] These peptides were desired to elucidate the structure–activity relationships of a tripeptide which shows such astounding hormonal activities at nanogram and picogram levels.

By this procedure, *N-tert*-butyloxycarbonyl-N^{im}-benzylhistidine (**2**)[5] was coupled with prolinamide (**3**)[6] by using *N,N'*-dicyclohexylcarbodiimide (DCI) to obtain in 92% yield the protected dipeptide, *N-tert*-butyloxycarbonyl-N^{im}-benzylhistidylprolinamide (**4**). Treatment of this dipeptide with glacial AcOH saturated with HBr to remove the *N-tert*-butyloxycarbonyl group gave a 72% yield of the crystalline N^{im}-benzylhistidylprolinamide·2HBr (**5**). This dihydrobromide was readily recrystallized for purification.

After drying the dipeptide·2HBr (**5**) *in vacuo* for 24 hr, it was coupled with *N*-carbobenzoxypyroglutamic acid[7] by the mixed anhydride method.[8] The resulting protected tripeptide, *N*-carbobenzoxypyroglutamyl-N^{im}-benzylhistidylprolinamide (**6**) was subjected to catalytic hydrogenolysis to remove the benzyl and carbobenzoxy groups to afford the pyroglutamylhistidylprolinamide (TRH) (**1**) in a yield of 61% over the last 2 steps. The chromatographic behavior, the spectroscopic properties, and the hormonal activities of TRH from this synthesis were identical with those corresponding characteristics of pGlu-His-Pro-NH_2 which have been described in the two companion papers.[1,2]

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