ACTIVITY AND SPECIFICITY OF SYNTHETIC THYROTROPIN-RELEASING HORMONE IN MAN

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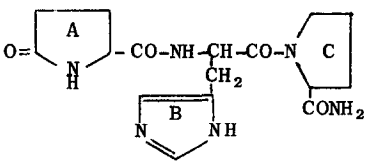
Summary. The serum levels of thyrotropin (TSH) increased two minutes after a quick single iv. injection of 800 μg of synthetic thyrotropin releasing hormone (TRH) into a normal male subject. The peak elevation of TSH occurred after 30 minutes, and was 370% higher than the level at 0 time; after 3 hours, the level decreased to normal. The levels of GH, LH, and FSH did not significantly change, but the plasma level of cortisol rose slightly. These studies show the hormonal activity and specificity of synthetic TRH to release TSH in man and indicate its future use for studying the pituitary-thyroid system of man in health and disease.

We have reported the isolation, structure, and synthesis of the porcine thyrotropin-releasing hormone (TRH)(1-5). These studies established the structure of porcine TRH to be (pyro)Glu-His-Pro(NH_2)(I). The cyclized glutamic acid moiety (A), the unsubstituted imidazole nucleus (B) and the prolinamide moiety (C) are probably critical for the extraordinary hormonal potency of this molecule since minor modifications of the chemical structure greatly decreased its hormonal activity (6-8). Studies performed concurrently by Burgus et al. (6) revealed that ovine TRH also has structure I.



The hypothalamic releasing hormones have occasionally been doubted, but sought for over a decade. Now that one of these hormones has been structurally elucidated and synthesized, their existence is established, in principle, and solving of the remaining hormones could be significantly expedited.

Synthetic and porcine TRH are active in vivo at a dose of one nanogram in mice (8), and stimulate the release of TSH in vitro from anterior pituitary glands of rats at picogram levels. The responses to TRH, in vivo and in vitro,

are inhibited by triiodothyronine. TRH will raise plasma levels of TSH in normal rats and mice and in hypophysectomized rats with pituitary transplants under the renal capsule. Inactivation of TRH occurs after incubation in human serum. In mice, TRH is active when administered ip., iv., sc., and orally.

Since porcine and ovine TRH are chemically identical, and since natural porcine TRH is active in mice, rats, and man (9-11), TRH appears to lack species specificity in many mammals.

At the recent American Thyroid Meeting (12), we reported that synthetic TRH does elevate levels of thyrotropin (TSH) in man. We have now extended the data on the hormonal activity of synthetic TRH in man and observed specificity of activity, since serum levels of growth hormone (GH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) did not change; the levels of plasma cortisol rose only slightly.

The synthetic TRH was dissolved in saline and the solution was passed through a millipore filter and stored in sterile vials. Serum levels of TSH, LH, FSH, and GH were measured in duplicate by radioimmunoassay. When a difference between duplicates was greater than 3%, the determination was repeated. Plasma levels of cortisol were measured by a fluorometric method (13).

Synthetic TRH was injected iv. into the ante-cubital vein of a 28-year old fasting normal male volunteer (72 kg.) within 20 seconds at 8:30 A.M. The patient was sitting upright. The times at which samples were taken before and after the injection of TRH are in Table I. During the control period, a blood sample was taken at minus 6 minutes; 2 ml. of normal saline was injected iv., and a second blood sample taken at 0 time. After taking the second blood sample, 800 μg of TRH in 2 ml. of normal saline was injected. There was no adverse clinical effect after the injection of TRH; blood pressure, pulse, and respiration were unchanged. Lower dose levels elicited a less significant response in other patients.

The results in Table I show that injections of saline had no effect on plasma levels of TSH; however, 2 minutes after injection of 800 μg. of synthetic TRH, the levels of TSH were significantly higher. The level of TSH was continuing to rise at 10 and 15 minutes after the injection. The peak elevation of TSH occurred after 30 minutes and was 370% higher than the level at 0 time. The levels of TSH at 30 and 60 minutes were almost the same; after 3 hours, the level had decreased to normal. Levels of GH, LH, and FSH at these time intervals did not change significantly. At the peak level of

TABLE I. SERUM LEVELS OF PITUITARY HORMONES AND CORTISOL BEFORE AND AFTER ADMINISTRATION OF SYNTHETIC TRH

Minutes	TSH	GH	LH	FSH	Cortisol
	ng/ml				μg/100 ml
-6	2.0	<1.0	0.6	1.9	16.5
0	1.2	<1.0	0.7	1.2	18.5
+2	2.4	<1.0	0.8	1.1	--
+6	2.0	<1.0	1.1	1.8	--
+10	3.5	<1.0	1.2	0.8	--
+15	4.5	<1.0	1.2	2.0	20
+30	4.9	<1.0	1.0	1.6	33
+60	4.8	<1.0	0.9	1.8	--
+120	3.0	<1.0	0.9	1.6	--
+180	1.9	<1.0	0.9	0.6	21

TRH, 800 μg, injected after the blood was taken at 0 time.

elevated TSH, the level of plasma cortisol had slightly increased. Previous studies (13) showed that after a quick single iv. injection of ACTH, plasma levels of cortisol peaked at 45 or 60 minutes while after iv. injection of 2 ml. of normal saline, at this time of day, the plasma cortisol levels were lower than the baseline level.

The initial administration of partially purified natural porcine TRH to human cretins indicated hormonal activity in man (10). On the basis of animal data with pure porcine TRH, it was calculated that 20 μg of pure TRH would have been necessary in such cretins to have elicited a response comparable with that of the impure sample. Cretins were previously thought to be hypersensitive (10). Judging from our current studies on normal male subjects, it is probable that cretins are more sensitive to TRH than are normal subjects.

This demonstrated activity and specificity of synthetic TRH in man forecasts that a combination of synthetic TRH and the radioimmunoassay of serum levels of TSH will become a valuable method for specifically investigating the pituitary-thyroid system of man in disease and health.

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