

Metabolism and Excretion of Exogenous Thyrotropin-Releasing Hormone in Humans

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ABSTRACT To study the metabolism of thyrotropin-releasing hormone (TRH) in vivo, 400 μ g TRH was administered intravenously to eight normal male subjects. Multiple plasma and urine samples were obtained before and after TRH administration. Serum TSH concentrations increased after TRH administration in all subjects. Plasma TRH levels, measured by radioimmunoassay, were undetectable (<0.4 ng/ml) before TRH administration. Plasma TRH concentrations averaged 33 ± 7 ng/ml (mean \pm SEM) 2 min after TRH injection. Thereafter, they decreased rapidly so that the mean plasma TRH level was 2.9 ng/ml 20 min after TRH administration. The fall in plasma TRH levels was linear during this interval. Thereafter TRH levels declined more slowly. The mean half-life ($t_{1/2}$) of TRH was 5.3 ± 0.5 min. The mean distribution volume was 15.7 ± 3.8 liters, an average of 16.5% of body weight in these subjects. In the urine, $5.5 \pm 0.9\%$ of the administered TRH was recovered in the 3 h after TRH administration. Of the total urinary TRH recovered, 84.9% was excreted in the first 30 min. These results indicate that TRH is distributed in a large volume, that it is rapidly metabolized and that a significant quantity of administered TRH is excreted in the urine.

INTRODUCTION

The administration of thyrotropin-releasing hormone (TRH)¹ to normal subjects is followed by a prompt increase in serum thyrotropin (TSH) concentrations. Peak TSH levels are found 20–30 min after TRH administration and they then decline rapidly (1, 2). These results, plus the observations that TRH is inactivated

rapidly by human plasma or serum in vitro (3, 4), suggested that TRH rapidly disappears from the circulation in vivo. The present study was undertaken to determine the magnitude of plasma TRH concentrations that follow its intravenous administration and its rate of disappearance and metabolic fate.

METHODS

Study protocol. Eight normal male subjects 17–32-yr old each received a bolus intravenous injection of 400 μ g TRH. None were receiving any medications. Six were normal weight and two were obese. None had any clinical evidence of thyroid disease, and all had normal serum T_4 , T_3 , and TSH concentrations (Table I). Blood was obtained for TSH and TRH determinations – 5, 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after TRH administration. 5-ml samples for TRH assay were collected in tubes containing heparin and 5.0 mg 2,3-dimercaptopropanol (BAL). These samples were immediately placed in an ice bath and centrifuged within 2 min after collection for 5 min at 2°C, and the plasma was frozen immediately. It previously has been shown (5), and further data is presented below, that BAL, in a concentration of 0.5 mg/ml plasma or higher, prevents TRH inactivation by serum at 37°C for at least 1 hr. Urine samples were collected for 60–120 min before and at 30–60-min intervals for 3 h after TRH administration. Immediately after collection of urine the volume was measured, and a portion was frozen.

Measurements. Serum TSH and T_3 concentrations were measured by radioimmunoassay (6, 7), and thyroxine was assessed by competitive protein-binding analysis (8). Plasma and urine TRH was measured by a sensitive and specific radioimmunoassay (9), all samples from an individual subject being assayed at the same time. The assay sensitivity at the time of these studies was 10 pg. Plasma samples were assayed in 10- and 25- μ l volumes since control plasma samples containing 1 mg/ml BAL assayed in these volumes contained no detectable TRH (<0.4 ng/ml). Larger plasma volumes containing this concentration of BAL nonspecifically inhibited binding of [¹²⁵I]TRH by anti-TRH serum. Recovery of TRH (10 ng/ml) added to 10 plasma samples containing 1 mg/ml BAL and incubated at 37°C for 1 h was $101.2 \pm 1.7\%$ (SEM).

The slope of the TRH disappearance line was calculated by the method of least squares, and the half time of disap-

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¹Abbreviations used in this paper: BAL, 2,3-dimercaptopropanol; TRH, thyrotropin-releasing hormone; TSH, serum thyrotropin concentrations.

TABLE I
Experimental Subjects

Subject	Age	Weight	Serum		Serum TSH	Max Δ TSH*
			T ₄	T ₃		
	yr	kg	μg/100 ml	ng/100 ml	μU/ml	μU/ml
R. Y.	23	57.1	6.6	78	<2.0	4.0
D. B.	24	84.3	5.0	68	3.6	13.3
B. F.	23	66.6	6.0	90	2.0	11.3
T. T.	23	60.0	7.6	98	<2.0	11.6
D. L.	25	74.3	5.6	68	<2.0	7.1
G. B.	27	76.2	8.7	98	3.6	13.0
W. F.	17	151.9	10.0	78	5.7	16.0
T. H.	32	160.2	6.0	74	3.2	19.8

* Maximum increase in serum TSH after TRH.
T₃, triiodothyronine; T₄, thyroxine.

pearance was calculated directly from the slope. The fractional turnover rate was calculated from the formula $\kappa = 0.693/t_{1/2} \times 100$. The zero time plasma TRH concentration was estimated by extrapolation of the disappearance line to zero time. From this, the volume of distribution was calculated (TRH administered/zero time TRH concentration).

The results of extensive studies of the serum inactivation of TRH immunological activity have been reported from this laboratory (4). However, most of these studies were done using a large quantity of TRH (2 μg/ml serum). When the plasma TRH results described herein became available, TRH, in a concentration (40 ng/ml) near that calculated at zero time (Table II), was added to eight normal plasma samples and portions were removed after varying intervals of incubation at 37°C. These portions were diluted 10-fold with 0.25% bovine serum albumin (BSA), 0.01 M PO₄, 0.15 M NaCl, pH 7.5, and their TRH content determined. The calculated rate of disappearance of this quantity of TRH in vitro was 4.1%/min, a value similar to that previously found in experiments in which the added TRH concentration was 2 μg/ml (6.2%/min) (4).

The effect of urine on TRH immunological activity was determined by incubation of TRH (20 ng/ml) in portions of fresh urine from five normal human subjects at 4, 22, and 37°C. Portions were removed for immunoassay before and

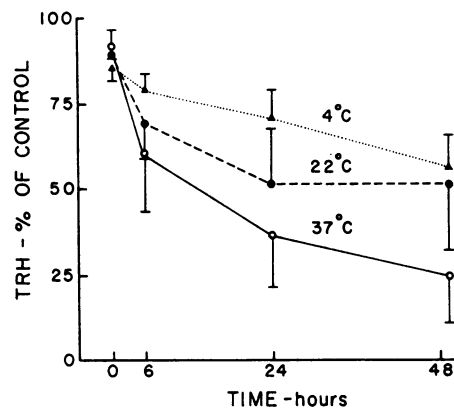


FIGURE 1 The effect of urine on TRH immunological activity. Urine containing 20 ng/ml TRH was incubated at the temperatures indicated and portions removed for TRH assay after varying intervals of incubation. Vertical bars indicate ± 1 SEM.

after 6, 24, and 48 h of incubation. As shown in Fig. 1, significant TRH loss occurred after 6 h of incubation at 22 and 37°C, but not at 4°C. After 24 and 48 h of incubation at all three temperatures, TRH recovery progressively declined. Because of these results, urine samples from subjects receiving TRH were not modified, though they were frozen as rapidly as possible after collection. Since some randomly collected urine samples in doses of 50–200 μl nonspecifically inhibited the TRH assay, all urine samples were assayed in smaller quantities. In the urine samples collected from 0 to 30 and 30 to 60 min after TRH administration, extensive dilution was needed for quantitation of urinary TRH.

RESULTS

TRH was readily demonstrable in plasma 2 min after intravenous TRH administration. Thereafter the plasma TRH concentrations declined rapidly (Fig. 2). The mean plasma TRH level was 33 ng/ml 2 min after TRH

TABLE II
Plasma TRH Disappearance and Urine TRH Recovery Results

Subject	Plasma TRH ₀ *	t _{1/2}	K _{TRH}	Dist. vol.	Body wt	Urine TRH recovered	Urine TRH recovered
	ng/ml					min	%/min
R. Y.	33	8.6	8.1	12.0	21.0	25.6	6.4
D. B.	24	4.4	15.8	16.4	19.4	18.2	4.5
B. F.	41	4.4	15.8	9.8	14.7	36.9	9.2
T. T.	62	5.2	13.3	6.4	10.6	10.3	2.6
D. L.	45	5.1	13.6	8.8	11.8	31.5	7.9
G. B.	61	3.9	17.7	13.2	17.3	15.6	3.9
W. F.	21	5.1	13.6	18.6	12.2	—†	—
T. H.	10	6.2	11.2	40.4	25.2	15.1	3.8
Mean	37	5.3	13.1	15.7	16.5	21.9	5.5
SEM	7	0.5	1.4	3.8	1.8	3.6	0.9

* Calculated plasma TRH concentration at zero time.
† Urine not collected.

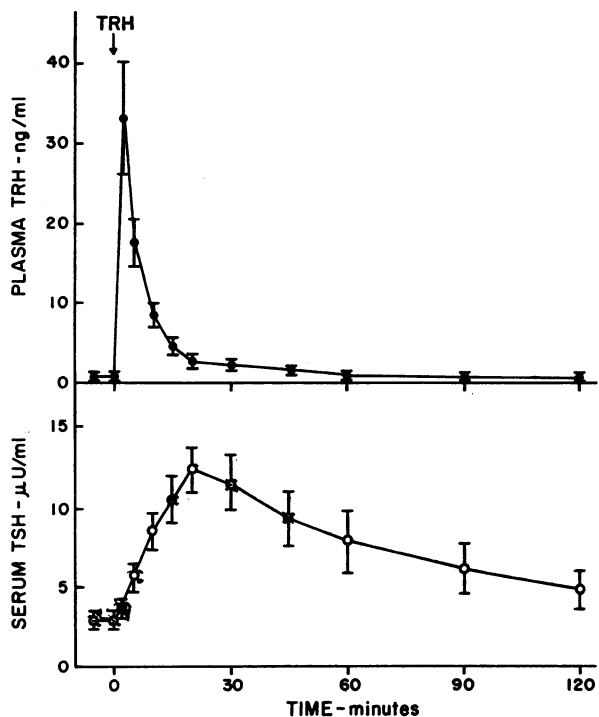


FIGURE 2 Mean plasma TRH (top) and serum TSH (bottom) concentrations after TRH administration in eight normal males. The vertical bars indicate ± 1 SEM.

injection, and it had fallen to 2.9 ng/ml 20 min after TRH injection. Thereafter the rate of decline was slower. The values found at 60, 90, and 120 min after TRH injection were all < 1 ng/ml. The increases in serum TSH concentrations, which occurred in all subjects and were, with one exception (R. Y.), within the range of normal previously reported from this laboratory for males in this age group (2), are shown at the bottom of this figure. There was no correlation between the peak plasma TRH and peak serum TSH concentrations. When the mean plasma TRH levels were plotted semilogarithmically (Fig. 3), the fall in plasma TRH appeared linear during the 2–20 min time interval. Therefore, the plasma TRH results obtained in this interval were used for the estimation of $t_{1/2}$. The results of the individual $t_{1/2}$, fractional turnover rate and distribution volume calculations are shown in Table II. The mean $t_{1/2}$ was 5.3 ± 0.5 min (mean \pm SEM) and the mean rate of disappearance $13.1\%/min$. The mean volume of distribution was 15.7 ± 3.8 liters, representing 16.5% of body weight.

TRH was not found in any of the urine samples collected before TRH administration. After TRH injection, TRH was readily detected in urine for 90 min, most being found in the first 30 min (Fig. 4). The overall recovery averaged $21.9 \mu\text{g}$ or 5.5% of the administered dose. Of this, an average of $18.6 \mu\text{g}$ (84.9% of the re-

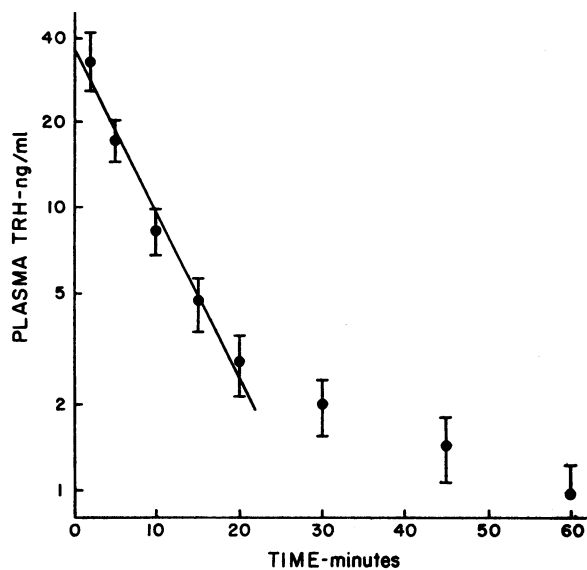


FIGURE 3 Semilogarithmic plot of disappearance of exogenously administered TRH. Vertical bars indicate ± 1 SEM.

covered TRH) was excreted in the first 30 min and $2.6 \mu\text{g}$ (11.9%) during the second 30 min after TRH administration.

DISCUSSION

These studies demonstrate that TRH disappears from the circulation very rapidly, over 90% being removed within the first 20 min after intravenous TRH administration. The variability of the $t_{1/2}$ results, from subject to subject, was not marked though in one obese subject (T. H.) low plasma TRH concentrations were found, which led to a high distribution volume estimate. The *in vitro* studies described suggest that the measured plasma and urine TRH concentrations were not artifactually reduced by inactivation of TRH after collection of the blood and urine samples. Thus, these results appear to give a true picture of the degradation of pharmacological quantities of TRH in man. In the only other report concerning TRH metabolism in human subjects, plasma

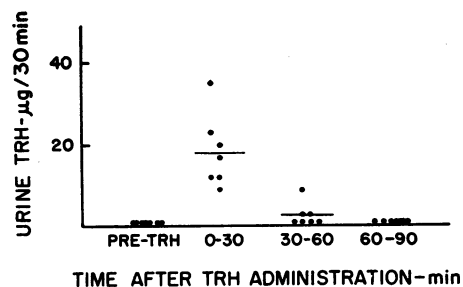


FIGURE 4 Urine TRH recovery after exogenous TRH administration.

TRH (bioassay) levels averaged about 8 ng/ml 1 min and 5 ng/ml 5 min after injection of 200 µg TRH in five subjects and substantial quantities of TRH were found in urine in the 30 min after TRH administration (10).

Several limitations of this study are evident. The half-life data presented were obtained from plasma TRH measurements after the administration of pharmacological quantities of TRH. The rate of TRH degradation at normal plasma TRH concentrations, which are <0.4 ng/ml, may be considerably different. Since endogenous plasma TRH measurements are not available, the data herein reported cannot be used to estimate the size of the TRH pool or the TRH secretory rate. Secondly, in this study TRH was injected into an antecubital vein whereas normally it is secreted into the hypothalamic-hypophyseal portal system. It is likely that the dynamics of TRH metabolism, especially in regard to the effect of the hormone on its target gland the anterior pituitary, may be different after hypophyseal portal rather than peripheral venous entry.

The calculated volume of distribution of TRH averaged 16.5% of the body weight. This value corresponds closely with current estimates of extracellular fluid volume in normal human subjects (11). The finding of a large TRH distribution volume is not unexpected in view of the small molecular size of TRH and the lack of evidence for binding of TRH to plasma protein (4, 12). The distribution volume result further suggests that there is little early intracellular penetration of TRH.

The rapid disappearance of TRH from plasma is undoubtedly a result of several metabolic processes. These include plasma and perhaps tissue destruction of TRH and renal excretion of TRH. TRH immunological activity is rapidly destroyed by incubation with plasma in vitro, and there is little reason to doubt the same occurs in vivo. The mean fractional rate of turnover of TRH in vivo, 13.1%/min, is roughly three times the rate of destruction of TRH by plasma in vitro. While it has been shown that homogenates of rat tissue destroy TRH biological and immunological activity (13, Bassiri and Utiger, unpublished observations), for reasons stated above it is unlikely that a substantial quantity of TRH enters cells. Administration of [¹⁴C]TRH and [³H]TRH to animals has been shown to result in tissue accumulation of radioactivity (tissue/plasma ratios > 1) in anterior pituitary, liver, and kidney but this radioactivity was not identified as TRH per se (14). It is possible even this radioactivity is not intracellular, but rather bound to the cell surface.

In these normal subjects, renal excretion accounted for 5.5% of the administered TRH, most of which appeared in the first 30 min after TRH administration. Thus at high plasma TRH concentrations, TRH is rapidly cleared by the kidney. Whether or not substantial renal clear-

ance occurs at endogenous TRH concentrations remains to be determined. In view of the rapid clearance of TRH, and the minimal inactivation of TRH which occurs in urine, urine TRH measurements may be more feasible than those in plasma.

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